National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1988

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Services
National Institutes of Health
National Institute of Dental Research

RK
80
N277
1988

FOR ADMINISTRATIVE USE ONLY

==

This compilation of the NIDR Annual Report is for administrative use only and is not to be released for non-Governmental purposes except under the Freedom of Information Act.

==

FOR ADMINISTRATIVE USE ONLY

CONTENTS

INTRODUCTION v

OFFICE OF THE DIRECTOR

Report of the Director (DIR) 1
Report of the Deputy Director (DIR) 7
Assistant Director for International Health (DIR IH) 11
Planning and Evaluation Section (OPEC PES) 17
Public Inquiries and Reports Section (OPEC PIRS) 21
Research Data and Management Information Section (OPEC RDMIS) 27
Financial Management Section (OAM FMS) 33
Personnel Management Section (OAM PMS) 35
Equal Employment Opportunity Program (EEO) 37

EXTRAMURAL PROGRAMS

Report of the Associate Director 43
Research Training and Manpower Development (RTMD) 47
Caries and Restorative Materials Research Branch (CRMR) 51
Craniofacial Anomalies, Pain Control and Behavioral Research Branch (CAPCBR) 69
Periodontal and Soft Tissue Diseases Branch (PSTDR) 83

INTRAMURAL RESEARCH PROGRAM

Bone Research Branch (BRB) 97
Cellular Development and Oncology, Laboratory of (LCDO) 117
Clinical Investigations and Patient Care Branch (CIPCB) 141
Diagnostic Systems Branch (DSB) 177
Developmental Biology and Anomalies, Laboratory of (LDBA) 189
Immunology, Laboratory of (LOI) 223
Microbial Ecology, Laboratory of (LME) 253
Neurology and Anesthesiology Branch (NAB) 271
Oral Medicine, Laboratory of (LOM) 299

EPIDEMIOLOGY AND ORAL DISEASES PREVENTION PROGRAM

Report of the Associate Director 317
Office of Program Support (OPS) 321
Microbial Systematics Section (MSS) 333
Analytical Studies Branch (ASB) 341
Disease Prevention and Health Promotion Branch (DPHPB) 353
Epidemiology Branch (EB) 369

INDEX BY PROJECT NUMBER 389

INDEX BY PRINCIPAL INVESTIGATOR 391

INTRODUCTION

The National Institute of Dental Research celebrated its 40th birthday in Fiscal Year 1988. It was a time to reflect on the scientific achievements of the past 40 years--advances that have brought the dental sciences into the mainstream of biomedical research.

In the four decades since NIDR became the third of the National Institutes of Health, toothlessness has been virtually eliminated among young and middle-aged adults. Today, half of all working adults in the United States aged 18 to 64 have lost at most one tooth, and only four percent are edentulous. This represents a significant drop in tooth loss even since the early 1970s. The news gets better--half of all children in this country ages 5 to 17 have never had a cavity, a remarkable improvement considering that almost all of their parents suffered from tooth decay as children.

While the younger segments of society have benefited greatly from 40 years of progress in dental science, older Americans have not been so fortunate. They continue to suffer high levels of caries, periodontal disease, and edentulousness. We must expand our research focus to address this ever-increasing population, lest we preserve the healthy teeth of our youngsters only to lose them later in life. Also warranting attention are high-risk individuals: the handicapped, institutionalized, immunocompromised, and others who are especially susceptible to dental disease.

As we celebrate our first 40 years, NIDR pledges its continuing commitment to promoting disease prevention and oral health among children, young and middle-aged adults, and older Americans alike to ensure that future generations never again experience the oral health problems of the past.

REPORT OF THE DIRECTOR

Research Directions and Policy

The 40th anniversary of the NIDR provided the Director with opportunities for retrospective and prospective views of dental research and its impact on dental education and practice. These occasions served as springboards for discussion of new directions and policies that might be initiated by the Institute to carry out its mandate of improving the oral health of the American people. In summary:

In 1948 and throughout the early years of the existence of the NIDR, the chief concerns of dental research were dental caries and fluoride studies. The prospect that the adjustment of community water supplies to achieve optimal levels of fluoride could lead to dramatic reductions in coronal caries in children created a wave of optimism in dentistry. For the first time in history there was hope that a disease that was endemic, painful and costly might come under control.

As the results of the first clinical trials comparing cities with and without fluoridated water supplies clearly established the efficacy of fluoride, the mood of optimism continued. Dental research turned to the disease process itself, establishing that decay was caused by bacteria in plaque. Microbiology and immunology studies expanded along with studies of nutrition, oral health promotion and disease prevention.

In the 1950s and 1960s dental research further expanded, producing definitive evidence that the periodontal diseases also were infectious conditions associated with different species of bacteria in plaque. Pioneering research was begun on connective tissue and bone and on hereditary diseases and congenital anomalies. To encourage nondental investigators to explore challenging questions in oral health, the Institute initiated broad-based dental research institutes and centers. This effort brought scientists with doctorates in biomedical and behavioral science fields to dental research in a process that has continued to the present. Today neuroscientists, endocrinologists, virologists, geneticists, epidemiologists, gerontologists, sociologists, psychologists and other health specialtists collaborate in studies of diseases and disorders of the oral tissues from AIDS to xerostomia.

This impressive growth in the depth and breadth of dental research has led to important changes in dental school curricula, resulting in an increased emphasis on the basic sciences and the incorporation of new methods of diagnosis, treatment and prevention of dental diseases and disorders. On their part, practicing dentists have witnessed major changes in the kinds of care needed and services provided. As recent NIDR epidemiologic surveys have indicated, the oral health of Americans has improved greatly in the course of 40 years, with the prospect that the majority of adults in future generations will retain their natural teeth over their lifetimes. To ensure that outcome, the Institute, through the Director, has developed policies and plans to address the oral health problems of all individuals and groups who may be at risk for oral diseases and, indeed, assure that research findings are rapidly communicated to the public and to practitioners for the benefit of all peoples everywhere.

Program Management: Research and Training Support Activities

As Acting Associate Director of the Epidemiology and Oral Disease Prevention Program (EODPP), the Director continued to advance research targeted to at-risk populations of adults and older persons, and directed development of NIDR monographs detailing regional findings from the National Survey of Oral Health in U.S. Employed Adults and Seniors and trends in dental caries among American schoolchildren. In related activities, the Director led the symposium on the status of oral health in the United States as a major NIDR 40th anniversary event.

The Director, serving as Acting Chief of EODPP´s Periodontal Disease Section, presented the periodontal disease findings from the most recent surveys of adults and seniors at the American Academy of Periodontology. He continues as senior investigator in an ongoing longitudinal study of the natural history of periodontal diseases; participates in the analysis of data on treatment needs for periodontal diseases based on the 1981 HRSA survey; and oversees development of periodontal disease survey measurement tools, training manuals and data analyses in all periodontal disease studies conducted in EODPP.

Extramurally, the Director initiated a Research Center for Oral Health in Aging and oversaw the issuance of RFAs (requests for applications) for a new specialized materials science research center, caries research center, and a craniofacial anomalies research center. The Director also participated in the Programs Advisory Committee meetings on pain/behavioral and salivary research. With the Office of Medical Applications of Research, he implemented a consensus development conference on dental implants and began plans for another on oral complications of cancer therapy. In the area of training, he defended and promoted research training initiatives, particularly the Dentist Scientist Award; supported an assessment of training needs which might be addressed by the NRSA mechanism; and chaired three meetings of the National Advisory Dental Research Council and the summary review of research and training.

The Director strengthened NIDR´s intramural efforts in AIDS research by creating a new Laboratory for Microbial Ecology (the search for a laboratory chief is underway) and by fostering NIDR AIDS Task Force meetings to report on research progress in all laboratories and branches, including EODPP and the Extramural Program. In other intramural activities, the Director participated in meetings of the Board of Scientific Counselors which reviewed favorably the Laboratories of Oral Medicine, and Cellular Development and Oncology. Progress has also been made in modernizing laboratory facilities and renovating animal quarters to meet AALAC accreditation, and a veterinarian has been hired.

Employee Opportunity

The Director reaffirmed the Institute s commitment to equal employment opportunity through a variety of activities and programs throughout FY 1988. The Director kept informed of EEO, affirmative action, civil rights and contract compliance issues through regular meetings with the Institute´s EEO Manager and fully supported the EEO Advisory Committee´s career enhancement activities, which led to increased NIDR applications to NIH training programs. The Director's encouragement of the recruiting of minorities, women and handicapped individuals for summer programs resulted in an employment roster of 55 percent women and nine percent minorities. In the NIH Summer Research Fellowship Program, minorities held 13 percent of the positions and women, 50 percent.

In related activities, the Director nominated women and minority representatives to the National Advisory Dental Research Council and Program Adivsory Committee; and provided funding support for, and staff participation in symposia, seminars and meetings focusing on minority careers in biomedical research. The Director also supported the development and distribution of a new pamphlet, "Research Training Opportunity for a Minority Post-Doctoral Candidate." This program, made available through a NRSA institutional training grant, is designed to produce competent investigators in laboratory and clinical biomedical research. A post-doctoral candidate from Howard University School of Dentistry in Washington, D.C. has been selected for this research training assignment.

Organizational Activities

In FY 1988, the Director initiated a five-phased organizational development intervention program to identify organizational issues/problems, develop action goals, identify activities to meet those goals, implement the activities, and assess the impact of the activities on issue resolution. The first phase, comprehensive data collection, has been completed with the assistance of a management consultant support services contract.

The Director established an NIDR ADP (Automated Data Processing) Coordinating Committee to develop standardized criteria and provide oversight for the procurement of office automation equipment and systems. The objective is to move to state-of-the-art compatible office automation to increase productivity and efficiency.

During this fiscal year, the Director orchestrated a reorganization of the Epidemiology and Oral Disease Prevention Program to facilitate development of new areas of dental epidemiology such as oral soft tissue lesions, craniofacial defects and pain; to provide a new focus on analytic studies of the impact of oral diseases on dental treatment needs, education and practice; and to emphasize the research and action program for disease prevention and health promotion.

The Director led Executive Staff in implementing NIDR operational guidelines for the new EPMS Performance Award System and added a mandatory supervisory element into all FY 1988 plans for SES/SSS/PMRS staff. In addition, the Director filled vacancies created by retirements in the Executive Officer and Budget Officer positions and continued review and implementation of safety activities.

The Director chaired regular meetings of Executive Staff and Small Staff (immediate OD directors) and periodically attended program staff meetings to assure awareness of management issues at all levels of the Institute and to be available to staff for open discussion.

Professional and Public Communications

The Director spent considerable effort addressing other Federal agencies to establish collaborative relationships on the new NIDR Research and Action Plan for Oral Health. Discussions also continued with the National Institute on Aging and the Veterans Administration on further implementation of the collaborative effort in geriatric dentistry. As host of the first World Health Organization (WHO) meeting on oral manifestations of HIV infection, the Director interacted with representatives of the Armed Forces, relevant DHHS components and the VA to discuss their respective efforts and how they may be linked to international collaborative networks.

During FY 1988, the Director met with visitors from abroad to explore collaborative efforts on various aspects of oral health research. These international visitors included representatives from the World Health Organization, Pan American Health Organization, United Kingdom, Australia, Canada, Norway and Sri Lanka.

The Director provided interviews and background materials to print and broadcast media, including the Washington Post, US News and World Report, Family Circle, Ladies Home Journal, and NBC affiliate WUSA in Washington, D.C. Topics of interest ranged from the results of the adult and children's oral health surveys to highlights of 40 years of research progress in the dental sciences.

The Director orchestrated a year-long series of activities related to the NIDR's 40th anniversary, which were designed to heighten the awareness of both the profession and the public about advances in dental research. Among these activities were a symposium, technical exhibits, and articles in professional journals as well as the lay press.

Honors and Awards

During FY 1988, the Director was awarded an honorary doctorate for scientific achievement by the Royal Dental College in Copenhagen.

Presentations

"Research in the Glorious Tradition." Second Symposium of Periodontics, Kos, Greece, October 1987.

"The 1985 NIDR Adult Oral Health Survey." American Academy of Periodontology, Denver, CO, October 1987.

"Oral Health of Adult Americans in 1985." U.S. Army Dental Training Conference, Garmisch, Germany, October 1987.

"The Best Is Yet to Come." University of Michigan/NIDR Dental Symposium, Ann Arbor, MI, December 1987.

"NIDR Priorities in Research." Ad Hoc Group for Medical Research Funding, Bethesda, MD, December 1987.

"U.S. Adult Dental Health Status--Impact on Dental Practice." University of California Dental School Dean´s Seminar, San Francisco, CA, February 1988.

"The Best Is Yet to Come." American Association for Dental Research (AADR), San Francisco, CA, February 1988.

"NIDR - And 40 Years of Progress in Dental Health." West Virginia University Dental School Annual Program of Research Reports, Morgantown, WV, March 1988.

Dental Science--Dental Health: New Frontiers Symposium, "Forty Years of Progress." AADR/IADR, Montreal, Canada, March 1988.

Address. Student Research Group, AADR/IADR, Montreal, Canada, March 1988.

"Forty Years of Progress--The Best Is Yet to Come." 24th Annual American Dental Association Dental Student Research Conference, Bethesda, MD, April 1988.

"Oral Health In Americans and Its Implications." University of Medicine and Dentistry of New Jersey, Newark, NJ, April 1988.

"NIDR: Past, Present and Future." AADR, Boston Section, Boston, MA, May 1988.

Dentistry in the Arts - "Opening Address." NIDR 40th Anniversary Celebration, Bethesda, MD, May 1988.

Symposium: Dental Science-Dental Health, "From Knowledge Base to Action Research." NIDR 40th Anniversary Celebration, Washington, D.C., May 1988.

"Research Impact on the Future of Dentistry." Pan-American Health Organization/Latin American Workshop, Washington, D.C., August 1988.

"Trends in Dental Disease in the U.S. and Abroad." Stetton Seminar, Stone House, Bethesda, MD, September 1988.

Publications

Loe, H. Oral health in U.S. adults: National findings. Chicago Dental Society Review 1988;32-35.

Loe, H. Dental science and dental health: Now and a century hence. J Dent Res 1988;76:797-798.

Loe, H. Periodontics of tomorrow. Dental Clinics of North America 1988;32:395-404.

Loe, H. Forword. In: Ekstrand, J., Fejerskov, O., Silverstone, L.M. eds. Fluoride in Dentistry. Munksgaard, Copenhagen, 1988.

Brunelle, J.A., Miller-Chisholm, A.J., Loe, H. Oral health of United States adults: Regional findings. NIH publication no. 88-2869.

Brown, L.J., Oliver, R.C., Loe, H. Periodontal diseases in the U.S. in 1981: prevalence, severity, extent, and role in tooth mortality. J Periodontol; in press.

Kingman, A., Morrison, E., Loe, H. Smith, J. Systematic errors in estimating prevalence and severity of periodontal disease. J Periodontol; in press.

Oliver, R. C., Brown, L.J., Loe, H. An estimate of periodontal treatment needs in the U.S. based on epidemiologic data. J Periodontol; in press.

Loe, H. 1988. The new preventive dentistry: An end to edentulousness. Phillip Journal; in press.

Loe, H. and Morrison, E. Epidemiology of periodontal disease. In: Genco R., Goldman, H., Cohen, D.W. eds. Contemporary Periodontics. St. Louis: The C.V. Mosby Company; in press.

Loe, H. Research on oral diseases and its impact on dental education and practice. Advances in Dental Research, in press.

Loe, H. "Today´s Dentistry - Based on Prevention, Driven by Research." Op-ed, 1988.

REPORT OF THE DEPUTY DIRECTOR, NIDR

The Deputy Director shares responsibility with the Director, NIDR, in the direction and management of the Institute's programs and activities, and has full authority to act on his behalf. With the Director, the Deputy represents the DHHS and the NIH on matters pertaining to the Institute's programs and budget, and ensures effective liaison with other Federal agencies, professional organizations, and the dental research community.

Institute Management

In addition to his regular responsibilities, the Deputy Director served for the months of May and June as the Acting Executive Officer, NIDR; for June as the Acting Budget Officer, NIDR; and for June and July as the Acting Director, NIDR. He was responsible for the recruitment and interview activities related to the appointment of the new Executive Officer, Ms. Yvonne du Buy, and assisted in the selection of the new Budget Officer, Ms. Earlene Taylor.

During this fiscal year, the Deputy Director served as the principal officer overseeing the conduct of a contracted study of office automation and management information systems of the NIDR, and held a similar role in the ongoing analysis and organizational development program being carried out by a management consultant team. The Deputy also assisted the Director in the reorganization of the Epidemiology and Oral Disease Prevention Program (EODPP) that was completed in FY 1988.

NIDR Advisory Council and Committee Activities

The Deputy Director served as the Executive Secretary of the National Advisory Dental Research Council and was responsible for the conduct of the January, May and September meetings, as well as a special July mail review meeting of the Council. The Deputy oversaw the committee management function of the NIDR, and in August recruited a new Committee Management Officer to fill the vacancy created by resignation. In other duties, the Deputy Director served as the Executive Secretary for the bimonthly NIDR Executive Staff Meetings and weekly Office of the Director small staff meetings. He also chaired the Senior Dental Ad Hoc Advisory Group overseeing the development of a congressionally requested research and action program for improving the oral health in adults and older Americans, and served as chairman of the NIDR Resources Review Committee.

NIDR and NIH Representation Activities

In the absence of the Director, the Deputy represented the NIDR at the NIH Bureau/Institute/Division meetings. Similarly, he represented the NIDR at the regular and special NIH Executive Officers meetings in the absence of the Institute EO. For the NIH, the Deputy Director served as one of two representatives to the PHS Personnel Performance Standards Review Board.

NIDR 40th Anniversary Activities

The Deputy Director participated actively in the planning and execution of the various 40th anniversary activities that occurred in FY 1988. He assumed primary responsibility for the organization and conduct of a special event held at the NIH as part of the anniversary observation, the American Dental Association Dental Student Research Conference. Invited to this three day conference were dental student representatives from schools in the U.S., Puerto Rico and Canada. The student participants were exposed to the research programs and scientists at the NIDR and attended a number of lectures designed to increase their interests in future careers in dental research. Financial support for the conduct of this conference was provided by an industry sponsor, the Warner Lambert Company.

Liaison Activities

The Deputy Director served as the NIDR liaison consultant to the National Affairs Committee of the American Association for Dental Research, the Council on Dental Research of the American Dental Association, and the Association of State and Territorial Dental Officers. Additionally, the Deputy maintained very close coordination with the Washington office of the American Dental Association, the American Association of Dental Schools, and the American and International Associations for Dental Research. The Deputy Director represented the NIDR by participating at the annual meetings of these associations as well as the annual conference of dental school deans meeting.

With the Director, the Deputy met with the administrator and senior officials of the Centers for Disease Control to establish formal collaborative agreement to participate in the adult health promotion action research program, including the detail of a CDC dental officer to this initiative. He also facilitated a similar detail of a dental officer from the Bureau of Maternal and Child Health and Resources Development of the Health Resource and Services Administration. On behalf of the Director, the Deputy Director led a team of NIDR officials to explore collaborative efforts with the National Centers for Health Services Research and Technology Assessment. The Deputy Director was also responsible for conceptualizing, developing, and negotiating agreement on the establishment, at the NIDR, of a three-year oral medicine--dental program management residency program with the personnel position for the resident to be made available by the Indian Health Service.

PHS Dental Activities

On November 1, 1987, the Deputy Director was appointed to an additional position of Deputy Chief Dental Officer of the PHS and promoted to the rank of Rear Admiral/Assistant Surgeon General in the PHS Commissioned Corps. In this joint position, he assists the Chief Dental Officer in providing leadership and coordination of PHS national and international oral health activities, and dental professional affairs of the Office of the Surgeon General; represents the Surgeon General to local, state, national and international groups and professional societies; provides advice and guidance on matters such as recruitment, retention, career development of PHS dental personnel; and performs other duties as assigned by the Surgeon General. On assumption of this appointment, the Deputy Director resigned his position of chairman of the Surgeon General Dental Professional Advisory Committee and has spent considerable energy in assisting in the oversight of dental activities of the PHS and carrying out a broad range of assignments.

Professional Activities

The Deputy Director maintains his academic appointment as a Professorial Lecturer in the Department of Community Dentistry, Georgetown University School of Dentistry. In this role, he conducts seminars and lectures and participates in other faculty activities. In 1988, he was appointed to the Editorial Review Board of the Journal of Dental Education. He continues as a special reviewer for the American Fund for Dental Health.

Presentations

"Career Pathways and Opportunities." COSTEP-PHS Commissioned Corps Meeting, Bolling Air Force Base, Washington, D.C., July 1988.

Seminar Leader. Harvard Robert Wood Johnson Dental Services Research Scholars Seminar, NIDR, Bethesda, MD, June 1988.

Welcoming Address. NIH Consensus Development Conference On Dental Implants, NIH, Bethesda, MD, June 1988.

"40 Years Of Putting Research Into Practice." Baltimore City Dental Society, Baltimore, MD, May 1988.

"A Commitment to the Future of the Dental Profession." Commencement Address, Case Western University School of Dentistry, Cleveland, OH, May 1988.

"The Impact of Dental Research on the Profession." Student Dental Research Group Awards Dinner, University of Maryland School of Dentistry, Baltimore, MD, April 1988.

"The Future of Dentistry." Keynote Address, Georgetown University Dental Alunmi Reunion and A.S.D.A. Clinic Day, Washington, D.C., April 1988.

Welcoming Address and Moderator. "Thank You Grand Rapids" Fluoridation Study Ceremony, Grand Rapids, MI, April 1988.

"The Challenge and Rewards of a Career in Dental Research." 24TH Annual ADA Dental Student Research Conference, NIDR, Bethesda, MD, April 12, 1988.

Welcoming Address and Conference Moderator. 24TH Annual ADA Dental Student Research Conference, NIDR, Bethesda, MD, April 1988.

"Advances in Dental Research." Bay Shore Dental Society Meeting at NIDR, Betnesda, MD, March 1988.

DHHS Program Update. Council of Deans, 65TH Annual Session of the American Association of Dental Schools, Montreal, Canada, March 1988.

"The NIDR at 40 - In Retrospect and Prospect." Washington Section, American Association for Dental Research, Rockville, MD, February 1988.

"A Changing Delivery System: Past, Present and Future--Public Perspective." 34th Annual Meeting of the Southern Conference of Dental Deans and Examiners, Nashville, TN, January 1988.

NIDR - Dental Schools Update. 29th Annual Conference of Dental School Deans, Captiva Island, FL, December 1987.

Moderator and Discussant -- Revitalization and Corps Issues. 22nd Annual USPHS Professional Association Meeting, Las Vegas, NV, November 1987.

"Dental Education in Transition -- Possible Scenarios." NIDR Clinical Staff Fellowship Conference, NIH, Bethesda, MD, November 1987.

"The NIDR: A Force for Change for Forty Years." 8th Annual New York-New Jersey Metropolitan American Association for Dental Research Meeting, School of Dental Medicine, State University of New York at Stony Brook, NY, October 1987.

Moderator, Session on New Frontiers and Special Problems. 50th Annual Meeting, American Association of Public Health Dentistry, Las Vegas, NV, October 1987.

ASSISTANT DIRECTOR FOR INTERNATIONAL HEALTH, NIDR

The Assistant Director for International Health is responsible for coordinating global oral research initiatives, ongoing programs and activities of the Institute, facilitating their progress and promoting communication among U.S. dental scientists with colleagues overseas. The Assistant Director for International Health has been designated as the PHS lead by the Chief Dental Officer in matters of international oral health and was awarded the PHS Superior Service Award in Fiscal Year 1988 for leadership in international oral health.

International Collaboration for Oral Health Research (ICOHR)

During this year, plans were made and the initial phase of a joint project with the Fogarty International Center was implemented to develop a research agenda for international collaborative opportunities for oral health research as well as training needs and sources of support for these efforts. Toward these ends, the World Health Organization (WHO) has helped us identify a special expert consultant, Professor Philip Holloway, Dean of the School of Dentistry, University of Manchester, UK, to assist in this major initiative. It is expected that a report of proposed research items and a catalog of resources would be made available during Fiscal Year 1989 and a workshop would be convened to obtain consensus on the final documents as well as to develop implementation strategies. The National Advisory Dental Research Council and the Fogarty Advisory Board have endorsed this project to contribute toward a research planning effort for the decade of the 1990´s.

International Collaborative Study of Oral Health Outcomes (ICS-II)

A sole-source contract was awarded by the NIDR to the Oral Health Unit of the WHO for the coordination of the U.S. replication in the WHO International Collaborative Study of Oral Health Outcomes (ICS-II). A sub-contract was awarded to the Center for Health Administration Studies of the University of Chicago for the coordination of the socio-dental data. During FY1988, the Assistant Director for International Health worked with the Contract Office to issue a competitive request for proposal (RFP) for the U.S. replication to be conducted in the Baltimore metropolitan and nonmetropolitan area. This contract was negotiated and awarded during FY 1988.

A meeting to coordinate international participation in this 10 nation study of industrialized and middle-income developing countries was held in Geneva, Switzerland in February 1988. NIDR technical assistance during the year was provided to facilitate specific participation of India (using India-U.S. Rupee Funds); Egypt (potentially using USAID country funds); and Israel (potentially using research grant funds from the National Institute on Aging and the U.S.-Israel Binational Science Foundation).

The Assistant Director traveled to Cairo to meet with the Egyptian investigators, The Minister of Health and U.S. Embassy-USAID staff to facilitate progress towards funding Egypt´s participation. On return to the

NIH, the Assistant Director met with the Minister of Health for Israel who was in attendance at the U.S.-Israel Symposium on Epidemiology and Aging, to obtain her support for the co-investigators´ participation in the Geneva meeting and the subsequent research grant proposal preparations. Correspondence between staff and Israeli researchers on this project is continuing to sharpen the proposal.

Coordination activities continue to be maintained with two other U.S. sites to be included in ICS-II: Indian Health Service (supported in part by the National Center for Health Services Research) and San Antonio, Texas (supported in part by the Centers for Disease Control).

Organizational Liaison: World Health Organization (WHO)

NIDR, as the WHO Collaborating Center for Epidemiology, Prevention and Treatment of Oral Diseases and Conditions, continued its close interactions with the Oral Health Unit of WHO in Geneva and with the Pan American Health Organization (PAHO) dental officer. Aside from ICOHR and ICS-II activities, NIDR hosted the first workshop on "Oral Manifestations of Human Immunodeficiency Virus Infection: Towards International Collaboration," held at the NIDR in March of this year. The purpose was to establish communications among all WHO centers working in this subject area and to identify international collaborative activities for U.S. agencies and organizations which could be incorporated into this newly formed network and to facilitate program implementation.

Over 50 participants discussed issues related to: 1) basic research and clinical studies; 2) health promotion and health education of the public and profession; 3) infection control; and 4) epidemiologic investigations and surveillance activities. Recommended initiatives were discussed and reviewed for implementation by the WHO Center coordinators at subsequent meetings held in March and in May. Further elaboration and decisions regarding appropriate and feasible division of tasks will be continued during the World Dental Congress in October 1988.

Informal meetings were held with the Director of the WHO Special Program on Research in Aging, a new resident program housed at the National Institute on Aging, to discuss potential international collaborative activities with respect to oral health research and older persons.

Support was given by the Institute on the merits of official relationships with WHO and the International Association for the Study of Pain. Given NIDR´s strength in research in this area and the involvement of our scientists in this organization, this appears to be a viable way of strengthening pain research capacity internationally.

Organizational Liaison: Federation Dentaire Internationale (FDI)

At the World Dental Congress held in Buenos Aires in October 1987, the Assistant Director chaired the FDI Working Group on Oral Health Promotion, participated in Executive Meetings of the Commission on Oral Health Research and Epidemiology, served as Consultant to the Scientific Programme Committee and was elected to membership on that body, and contributed to the planning of an NIDR 40th Anniversary session for the FDI Congress to be held in Washington, D.C. in October 1988. The Assistant Director also will chair a session on Improving Access to Oral Health Care at the same Congress and will coordinate a session on Communications for the Congress to be held in Amsterdam in 1989.

Subsequent to the meeting in Argentina, the Assistant Director and the Director of the NIDR provided guidance for the Terms of Reference to be used for the Joint FDI/WHO Working Group on AIDS. The Assistant Director was invited to serve on the FDI Committee which selects the Johnson and Johnson Preventive Dentistry Awards.

At the 1988 FDI World Dental Congress, NIDR has initiated plans to provide tours for visitors, a special exhibit at the Smithsonian featuring 40 years of dental research, and a special colloquium on the impact of dental research on dental practice.

Organizational Liaison: International Association for Dental Research (IADR)

During the annual meeting of the International Association of Dental Research held in Montreal, the Assistant Director organized an NIDR 40th Anniversary symposium entitled, "Dental Science-Dental Health: New Frontiers." It successfully covered research highlights over 40 years, a historically-oriented film documentary of NIDR, papers on new research frontiers and their impact on dental education. The American Association of Dental Research (AADR) Committee on New Frontiers is working with the NIDR to contribute these papers on prognoses for dental research to the long-range research planning effort for the decade of the 90´s.

Organizational Liaison: American Dental Association (ADA)

President James Saddoris appointed the Assistant Director to an ADA Committee on Global Oral Health to advise the Board of Trustees on new directions in international health. Two formal meetings were convened at the ADA headquarters in Chicago, several informal meetings of committee members were held in Bethesda and considerable correspondence and drafting of report language transpired. The final report and recommendations were submitted to the Board in August 1988.

Cooperation with ADA staff continued during the year, particularly with respect to plans for the 1988 World Dental Congress, FDI organizational reports, the National Council on International Health, of which the ADA is a charter member, and information referrals for dentists seeking international opportunities in research. The Assistant Director serves as a consultant to the ADA Council on Scientific Programs and International Relations.

Organizational Liaison: **Fogarty International Center (FIC)**

The Assistant Director represented the NIDR at the BID International Representatives meetings held every six weeks and coordinated by the FIC; submitted the NIDR annual international report contribution toward the NIH annual document; transmitted NIDR comments on a variety of circulated documents including WHO, PAHO and Western Pacific Regional Office plans and reports, provided information on proposed FIC international fellows; attended FIC Advisory Board meetings held three times annually; served on the advisory committee on the FIC contract dealing with international health research expenditures data comparability system and cooperatively planned and directed the new initiative, International Collaborations on Oral Health Research (ICOHR).

The Fogarty International Center staff assist in furthering NIDR collaborative projects under the U.S.-Mexico, U.S.-India and U.S.-Italy agreements. As part of the NIH agreement with The Center for Research and Advanced Studies in Mexico, the NIDR intramural program in pain research has been exchanging scientific expertise in neuroanatomy. Under the auspices of the India-U.S. Science and Technology Agreement, rupee funds are being used to assure India´s participation in ICS-II. Other research activity related to fluorosis, betel quid and microbiological properties of plants have been discussed. Under the U.S.-Italy Science and Technology Agreement, NIDR bone researchers and investigators at the University of Rome are studying osteonectin, and a new proposal on bone research is under consideration with investigators at the University of Bari.

Interest has been expressed by the Taiwanese National Science Council in areas of cooperation in dental science. The Assistant Director met with Council representatives and discussed possible areas of interest described in "Challenges for the 80´s: NIDR´s Long-Range Research Plan FY 1985-1989. This meeting was hosted by the FIC.

NIDR travel to the AIDS Conference in Stockholm was coordinated with the FIC and the NIH AIDS Coordinating Committee. Seven NIDR staff attended this major meeting. Other NIDR staff travel abroad was reviewed for appropriations by the Assistant Director.

Other International Activities

During this fiscal year, the Assistant Director coordinated staff reviews on applicants seeking support from the U.S.-Israel Binational Science Foundation, provided orientations to international visitors from Australia, Great Britain, Mexico, Norway, People´s Republic of China, Saudi Arabia, Scotland, and Sri Lanka; and provided written responses to inquiries from Australia, Great Britain, The Federal Republic of Germany, Japan, The Netherlands, Nigeria, Norway, South Africa and Sweden. Requests for information ranged from possible sabbatical appointments at the NIDR to specific commentary on proposed or ongoing research.

The Assistant Director coordinated Institute sponsored participation in the World Conference on Health Education held in Houston, Texas in August 1988 which focused on oral health promotion initiatives for the U.S. and in other industrialized and developing countries. An important evaluation project related to international research performance using bibliometric and citation analysis is underway in the Planning and Evaluation Section of OPEC, which will guide planning of future international collaborative research efforts of the Institute.

The 1988 Percy T. Phillips Visiting Professorship at Columbia University School of Dental and Oral Surgery, and supported by the New York State Dental Society, was awarded to the Assistant Director. Her lecture focused on international collaborative opportunities in research, education and service, and will be published in the The Journal of the New York State Dental Society. An article summarizing much of this material was prepared for the Newsletter of the National Council on International Health.

Presentations

"Overcoming Cultural Barriers in Developing Countries." Fourth Annual International Conference of Chief Dental Officers, Buenos Aires, Argentina, October 25, 1987.

"Advances in Dental Research: The National Institute of Dental Research in 1987." Golden Jubilee of the Egyptian Dental Association and the Third International Congress, Cairo, Egypt, November 1987.

"Global Oral Health and Opportunities for International Collaborations." Percy T. Phillips Memorial Lecture at Columbia University School of Dental and Oral Surgery, New York City, N.Y., June 1988. Scheduled for publication in The Journal of the New York State Dental Society.

Seminars: Columbia University School of Dental and Oral Surgery, Percy T. Phillips Visiting Professorship, New York, New York. June 1988:

"Social Science Research in Dentistry: Contributions to Dental Health and Dental Practice."

"Impact of Psychosocial and Cultural Factors on Dental Disease and Dental Care in Different Countries."

"Relationship Between Dental Needs and Demand: Converting Unmet Needs for Care in Different Countries."

OFFICE OF PLANNING, EVALUATION AND COMMUNICATIONS (OPEC)

OPEC directs and coordinates all scientific program planning as well as all information necessary to support budget submissions, Congressional testimony and related documents which reflect NIDR's program goals and objectives. It is also responsible for coordinating and directing all program evaluation activities, assessing the impact of and return on NIDR's investments in research and training. OPEC prepares responses to inquiries from congress, other Federal and nonfederal agencies, the professions and the lay public; and collects appropriate materials and data to respond to such inquiries and to disseminate the informational outcomes of research to potential user organizations and communities. This Office often provides orientation services to visitors, new staff and consultants; organizes NIDR seminars on issues related to science policy; and provides essential support services to the Director of the Institute in developing documentation for speeches, presentations and publications.

PLANNING AND EVALUATION SECTION (PES)

The Planning and Evaluation Section, OPEC, coordinates all planning and evaluation activities for the NIDR, originates special planning initiative projects, and responds to internal (NIDR) and external requests for information relevant to planning and evaluation. Planning and evaluation functions are coordinated with both budget and information systems in concert with operating programs of the Institute. PES also serves as NIDR contact with the Division of Legislative Analysis, NIH.

Planning and Evaluation Activities

Staff was involved in several preliminary activities for developing the NIDR "Long-Range Research Plan for the Nineties." Staff organized and conducted the initial meeting of the NIDR "Plan for the Nineties" Coordinating Panel, November 1987; obtained background information for the Expert Panel meetings, to be held in early FY 1989, through responses to a notice placed in the Federal Register; and recommended tentative members of Expert Panels to the Director, NIDR.

Staff developed and coordinated the annual research planning session with the Director, NIH; the NIDR component of the NIH Research Plan; and the NIDR FY 1989-1990 Evaluation Plan. PES worked with the Director, NIDR and the Budget Office in preparing the FY 1989 Budget Justification narrative and opening statements by the Director for the House and Senate Appropriations Subcommittee hearings, coordinated responses to questions submitted for the record, and provided text for the FY 1990 Professional Judgment Budget. Staff worked with the Director in planning, writing, and editing formal presentations before a variety of audiences and written materials for professional journals.

PES, working with representatives of the National Institute on Aging and Veterans Administration, implemented collaborative research on oral health in the aging through the establishment of a research center. Staff assisted in preparation of the 1987-1988 NIDR Biennial Report of the Director and of the

National Advisory Dental Research Council, and developed a summary booklet, "NIDR - 40 Years of Progress." Staff helped draft a Congressional Resolution on NIDR´s 40th anniversary and a Presidential Proclamation. Staff worked with various dental research, educational and professional organizations in connection with responses to NIH and Congressional initiatives and in planning meetings.

Ongoing evaluation research projects include an assessment of NIH, industry and academic relationships in restorative materials research; a bibliometric evaluation of NIDR research; and the development of indicators for comparing the dental research activities of countries and institutions throughout the world. Staff submitted proposals for two new set-aside evaluation projects for FY 1989-1990; one would assess the Dentist Scientist Award, while the other would establish baseline data for monitoring the international dental research performance of institutions and countries according to the research areas specified in Challenges for the Eighties.

Other Activities

Staff served as a member of the following groups or committees:

NIDR AIDS Task Force
NIA-NIDR-VA Collaborative Project on Oral Health in Aging
NIDR Planning Committee for the 1989 National Health Interview Survey
NIH Evaluation Course Planning Committee
NIH Evaluation Technical Review Committee
National Cancer Institute Technical Review Committee
Evaluation Project Advisory Committee, National Library of Medicine for "TOXLINE Evaluation."

Presentations

Jim Lipton

"Evaluating the NIDR Long-Range Research Plan for the 80s, Challenges for the Eighties." Meeting of the NIDR Coordinating Panel for the Long-Range Plan for the Nineties, Bethesda, MD, November 1987.

"Comparison of Research Performance Among U.S. Dental Education Institutions." Annual meeting of the American Association of Dental Schools, Montreal, Canada, March 1988.

"International Comparison of Dental Research Performance." Annual meeting of the American Association for Dental Research, Montreal, Canada, March 1988.

"The Diagnosis and Treatment of Craniofacial Pain." Southern Maryland District Dental Society, March 1988.

"Dental Manpower Model for the Commissioned Corps." U.S. Surgeon General and staff, and various committees of the U.S. Public Health Service, 1987-1988.

"An Evaluation of the NIDR Small Grant Program." National Advisory Dental Research Council, Bethesda, MD, September 1988.

Joan Wilentz

"History of the Development of Challenges for the Eighties." Meeting of the NIDR Coordinating Panel for the Long-Range Plan for the Nineties, Bethesda, MD, November 1987.

PUBLIC INQUIRIES AND REPORTS SECTION

The Public Inquiries and Reports Section conducts a comprehensive information program using a variety of communications mechanisms. Research advances in the oral health sciences are shared with the public, the Congress and the dental profession through the development and distribution of patient and professional education materials, publications, exhibits, scientific reports, films, and extensive interaction with the trade and lay print and broadcast media.

40th Anniversary

PIRS staff negotiated with the managers of NOVA, the American Fund for Dental Health, and Barbara Holocek Productions and established a collaborative agreement to produce a major documentary on dental science. Staff coordinated communication with corporate sponsors, supervised the production schedule, and provided advice and technical assistance.

PIRS planned and coordinated a major commemorative program in Grand Rapids, Michigan to officially recognize the city's pioneering fluoridation efforts. Program participants included the NIH director, present and past NIDR directors and other major national, state and local dental officials. In conjunction with this program, PIRS wrote and produced a special videotaped message from the Surgeon General, compiled a videotape of historical footage of the Grand Rapids project, and developed a formal commemorative scroll for presentation to the city. Staff prepared a press kit and made advance and onsite media contacts that resulted in broad coverage of these activities.

Information staff initiated and coordinated local 40th anniversary activities, including a lecture on "Dentistry in the Arts" and a symposium entitled "Dental Science-Dental Health: The Status of American Oral Health." In conjunction with the symposium, staff coordinated, with the American Association for Dental Research, a reception and formal dinner, involving development of the guest list, site selection, and invitations. PIRS also coordinated the development and design of a *bas relief* of Dr. H. Trendley Dean and arranged the program for the dedication of the Bldg. 30 conference room to his memory. PIRS developed all supporting material such as posters, invitations and advanced publicity for these activities.

PIRS developed a special 40th anniversary poster, commemorative folders and a variety of related materials for use and distribution at the NIH and at major dental meetings throughout the year.

Staff submitted major 40th anniversary articles highlighting the history and accomplishments of the NIDR to the *Journal of the American Dental Association*, *Journal of Dental Research*, *Public Health Reports*, and *Dental Hygiene*. PIRS also prepared an op ed article, under the Director's signature, on advances in dental research for distribution to 600 daily newspapers nationwide.

PIRS staff provided extensive articles on the history and research advances of the Institute for a special commemorative issue of the "NIH Record." Information staff also arranged for the production of five articles, in conjunction with the 40th anniversary, which were sent to all weekly and small daily newspapers on dental advances.

PIRS researched, wrote and produced a historical videotape and film for viewing at major professional meetings of dental practitioners and researchers, and at NIDR anniversary functions. Videotapes were also distributed to all U.S. dental schools.

PIRS coordinated the Institute´s anniversary activities at the International Association for Dental Research General Session in Montreal, including a historical exhibit, 40th anniversary symposium, reception, and viewing of the historical film.

PIRS staff continued to direct and assist the research, writing and editing of a historical monograph on the NIDR, served on the review committee, and negotiated with a publisher concerning the printing of the manuscript. The 500-page history was completed and illustrated during FY 1988, with publication anticipated in the next fiscal year.

Staff cooperated with the American Dental Association (ADA) in arranging a Congressional breakfast on Capitol Hill with members of the appropriations committees, and in ADA´s efforts to prepare a Congressional joint resolution recognizing 40 years of dental research.

Staff assisted in planning the program, and arranging and conducting tours of the NIDR intramural research program for the ADA Dental Students Research Conference.

PIRS collaborated with the National Library of Medicine on a commemorative exhibit, "A Century of American Dental Research." An illustrated booklet, developed with assistance from PIRS, accompanied this exhibit.

Staff prepared a major commemorative Institute publication featuring articles on NIDR history and current research advances for distribution at the World Dental Congress to be held in Washington, D.C in October 1988.

In conjunction with the World Dental Congress, preparations are underway for a variety of projects including: special postal booth and commemorative cancellation, a symposium, tour of the NIDR facilities at NIH, and a viewing of the dental research documentary. Plans are underway in PIRS for the production of an exhibit on the history of the Institute to be displayed in the Smithsonian during this international meeting Staff participated in negotiations to secure museum facilities for a reception marking the opening of the exhibit.

Public and Professional Education

During FY 1988, PIRS carried out the following activities in the areas of public and professional education: directed preparation of press summaries on NIDR-supported research advances for the American Association for Dental Research/International Association for Dental Research meeting in Montreal; prepared bimonthly issuances of the "NIDR Research Digest" for inclusion in the IADR newsletter; provided articles for publication in the NIH research advances section of the Journal of the American Medical Association; and contributed articles on recent research advances to the Journal of the American Dental Association for the continuing NIDR series.

During this fiscal year, staff also distributed four dental health articles to the nation's small newspapers (dailies and weeklies), prepared articles on dental health for publication in the syndicated "NIH Search for Health" column, contributed regularly to the "NIH Record" and "NIH News and Features," and continued periodic publication of "NIDR Research News."

In related activities, PIRS staff provided background material for articles on NIDR research efforts to professional journals and health-oriented publications including the Journal of the American Dental Association, Dental Health Advisor, American Health Magazine, Dentistry Today, Dazzle, Dentists and Men's Health Magazine. Staff also prepared an article for International Health News and other publications in the field, and provided articles on pain for the December 1987 and January 1988 issues of the Journal of Practical Nursing.

PIRS staff served on the planning committee for the NIDR Consensus Development Conference on Dental Implants, and arranged all publicity for both the profession and the public. These activities included the development of posters, tent cards and registration forms; preparation of "Federal Register" and "Calendar of Events" notices, and "NIH Record" story; notification of the media for the press conference, and facilitating media access to conference participants. Following the conference, PIRS coordinated the administrative aspects of publication of the proceedings. Staff also serves on the planning committee for the NIDR Consensus Development Conference on the Oral Complications of Cancer Therapy, scheduled for FY 1989.

PIRS planned, coordinated and directed all arrangements for the 1988 Seymour J. Kreshover Lecture, "Bacterial Adhesion to Oral Tissues: A Model for Infectious Diseases," delivered by Dr. Ronald J. Gibbons of Forsyth Dental Center.

PIRS coordinated publicity for the NIH G. Burroughs Mider Lecture presented by an NIDR intramural investigator. Activities included the preparation of an article for the "NIH Record" and introductory comments for the Director, NIH, as well as the design and production of a poster and tent cards.

Last fiscal year, staff negotiated a joint agreement between NIDR and People's Drug Stores to prepare, at no cost to the Institute, a pamphlet promoting oral health. Staff provided background materials, a detailed outline, and a final review of the publication. In FY 1988, "Keep Your Teeth for a Lifetime" was published and 80,000 copies were distributed throughout the People's Drug Store chain.

PIRS exhibited at the annual scientific sessions of the International Associaton for Dental Research/American Association for Dental Research, the American Association of Dental Schools, and the American Dental Association. In conjunction with NIH's 100th anniversary, staff exhibited and participated in the NIH Open House weekend activities.

PIRS duplicated and distributed a slide-tape presentation by NIDR clinic staff on periodontal disease for use by health care providers. Staff also consolidated, into a single videotape, highlights of NIDR research news stories aired on local and network television. The tape was made available for viewing at NIDR staff seminars.

PIRS effectively orchestrated a multi-media patient recruitment effort for the NIDR clinical research program in the areas of pain, herpes, and other oral health studies.

Publications

Staff worked extensively with NIDR epidemiologists to prepare a monograph for publication on the regional findings of the adult oral health survey. Also in progress are an orientation handbook for new NIDR advisory council members, a pamphlet for patients on temporomandibular joint dysfunction (TMJ), and an intramural research activities booklet.

PIRS staff edited an article on Sjogren´s syndrome for FDA Consumer; and prepared and edited final copy, in collaboration with the National Institute on Aging, for an "Age Page" on oral health care for the elderly.

The following publications were updated, rewritten and reprinted in FY 1988:
"Periodontal (Gum) Disease,"
"Dentist Scientist Award,"
"Graduate Training Supported by the National Institute of Dental Research,"
"NIDR, Intramural and Extramural brochures.

Media

During this fiscal year, PIRS arranged interviews and provided background information on dental research topics to numerous general circulation magazines, including Parenting Advisor, Forbes Magazine, Better Homes and Gardens, Family Circle Magazine, Cosmopolitan, and Vogue; to the trade press; and to virtually every major metropolitan newspaper, news magazine and wire service, including the New York Times, Toronto Globe and Mail, Los Angeles Times, Washington Post, Newsweek, Associated Press, United Press International, Reuters, New York Daily News, High Technology Business Magazine, ABC News, Boston Globe, Newsday and Education Daily. Requests for radio interviews were received from National Public Radio, Iowa Public Radio, Voice of America, CBS Radio and FM96 News in Montreal.

PIRS prepared a press release on an intramural research finding involving the AIDS virus and saliva, and coordinated media activity which resulted in coverage in virtually all major newspapers. To date, over 400 press clippings from around the country have been returned. Similarly, press releases prepared by PIRS on NIDR advances in cancer metastasis and the development of a new laboratory tool for cancer research generated extensive coverage nationwide.

PIRS staff prepared a press kit and directed a major press conference at the NIH to announce the results of the National Survey of Oral Health in U.S. Schoolchildren: 1986-1987. Both print and broadcast media featured the story prominently throughout the country, resulting in the most extensive coverage an Institute-supported research advance has ever received.

Reports

PIRS contributed to seven Special Reports to Congress, including research advances in the following areas: cystic fibrosis, diabetes, arthritis, degestive diseases, AIDS, maternal and child health, and international activities. In addition, staff prepared the NIDR Director´s Report for the NIH Biennial Report, 1987-1988.

General Communications Activities and Services

Staff developed major speeches on dental research advances for the NIH Director, DHHS Assistant Secretary for Health, and Congresswoman Constance Morella.

PIRS responded to approximately 9,000 requests for information on a broad range of topics from the public, professionals, Congress and the media. Over 550,000 publications were sent out during FY 1988.

Staff provided medical arts, photography, graphics and printing services to the Institute for activities such as the EEO Bulletin and the NIDR Awards booklet. PIRS also arranged the production and printing of health promotion materials for the Science Transfer and Research Analysis Branch.

In FY 1988, PIRS let a year-long contract for a clipping service to monitor NIDR-supported research activities reported in the press. Staff also initiated negotiations to establish a contract for exhibit services.

Information office coordinated manuscript and abstract clearance through OD; arranged for review and clearance, by Institute experts, of articles prepared by the lay press; and directed the Institute´s contract mailing and storage operations with St. Elizabeth´s Hospital.

Other services included arranging a series of presentation skills seminars for NIDR staff to enhance public speaking abilities; providing resource material for the "NIDR ON-LINE" communications system; coordinating Institute submissions to the NIH Scientific Directors and Annual Bibliography; and coordinating exhibit scheduling and arrangements for the Science Transfer and Research Analysis Branch.

Publications

Sheridan P. Diabetes and oral health. J AM Dent Assoc 1987;115:741-2.

Sheridan P. Peptide blocks tumor cell invasion. J Am Dent Assoc 1988;116:538.

Sheridan P. National Institute of Dental Research: 40 years of progress. J Am Dent Assoc 1988;116:837-44.

Sheridan P. NIDR: 40 years of research advances in dental health. Public Health Rep 1988; in press.

RESEARCH DATA AND MANAGEMENT INFORMATION SECTION (RDMIS)

This Section of the Office of Planning, Evaluation, and Communications (OPEC) in the Office of the Director, continues to be the central source of administrative information about dental research supported by this Institute. Predominately computer oriented, the day-to-day activities consist of collecting and processing the various elements of information about research and research training grants, contracts, and intramural projects supported by this Institute for eventual retrieval and compilation into various routine and special reports and publications. Staff members are constantly in pursuit of new techniques and equipment to improve their skills and willingly lend this knowledge to others in their working environment.

Research Projects Management System Updated

Throughout the years, the RDMIS has provided the Grants Management Office, Budget Office, and Executive Office with up to the minute reports on the Institute´s financial expenditures, budget allocations, grant commitments, etc. These reports have been an excellent source of information and have served these individuals and their respective sections well. Recently, however, key personnel from each of these sections have retired and new officials have been hired to fill the vacant positions. But with new individuals come new and bright ideas, and new information they need at their fingertips. As a result, the RDMIS has been extremely busy re-designing their Research Projects Management System to meet the information needs and reporting requirements of the new personnel.

Calculation of Percentile Numbers

The addition of several new data items and changes in reporting concepts have also resulted in substantial system modifications. One new data item in particular involved the addition of the Percentile Number calculated for the IRG Priority Score. The percentile number represents the relative position or rank of the priority score among all priority scores assigned by the IRG. It is quickly gaining popularity among many of the BID´s as a funding mechanism. While NIDR is still basing their funding decisions on Priority Scores, the Percentile Rank will eventually replace this current method. Consequently, the RDMIS, in anticipation of this change and at the request of several Program Officers, has incorporated the Percentile into their Database, Dictionary, and several reporting programs.

Impact of Reorganization

Another structural change in the NIDR has had extensive ramifications in the reports we produce. This is the creation of "AREA 8: Salivary Research". Once again, our data files, dictionary, and reports were re-programmed to accomodate this modification. In addition, our Budget reports needed revising on several occasions due to the new expanded CAN structure to track AIDS expenditures in Intramural and Extramural programs.

Technical Reports

Fiscal Year data are compiled and printed in a series of technical reports that serve as handy references and provide the Institute with and archival record. The current list includes, NIDR Programs, NIDR Indexes, Selected List of Technical Reports, and Trainees and Fellows Supported by the National Institute of Dental Research. The NIDR Annual Report, required by the Deputy Director for Intramural Research at NIH, is still coordinated by the Section Chief but is much more restricted in its distribution.

NIDR ONLINE

In order to tighten the lines of communication between the NIDR and our constituent dental research community, we developed a menu-driven computer program called NIDR ONLINE in FY86. The program makes use of a number of existing databases compiled and maintained by NIDR for its own use--directories; lists of films; posters and publications; recent awards; lists of grants and contracts--that we thought might have some value to a larger audience. We then added some items like fact sheets; news; program announcements; conferences and seminars; and similar categories of potential interest to our users. Finally we added a one-way message option that allows our users to order films and publications or to send messages to anyone at NIDR.

Originally intended for dental research libraries, and over 50% of them are registered users, we soon discovered that the individual scientists were more interested in direct access from their own offices, laboratories, or homes. Almost anyone with a computer, modem, and communications software can access the system directly or through TYMNET from any place in the United States or Canada. Other networks permit access from any place in the world.

We now have over 170 registered users and the number continues to grow. In the last year, we had 236 users logon to the system and who selected 629 menu options to scan, read or copy for others. In an effort to promote the NIDR ONLINE facility to our constituent audience, a portable terminal was set up to demonstrate its potential value at the annual meetings of the American Association of Dental Schools, the International and American Association for Dental Research, and the Medical Library Association.

DENTALPROJ

After several years of negotiation and planning, we signed a Letter of Agreement in April with the National Library of Medicine to add a small public file to the MEDLARS system to be called DENTALPROJ. The purpose of the file is to expedite the transfer of information about dental research projects that are in progress -- that is before they are completed and before the results are published and indexed and otherwise available to the general scientific community.

The information available online will include the title of the project; name of principal investigator; performing institution; sponsoring organization; brief abstract of the project; and all of these data elements will be searchable by NLM´s Medical Subject Headings. It will include all of those projects of interest to dental scientists supported by the National Institutes of Health and efforts have been made to include similar projects supported by the Department of Defense, the Veteran´s Administration, and all others that can be collected.

Of special value to NIDR will be the ability to search these projects in greater depth, to search more broadly, and to search them more quickly than we have ever been able to do in the past. This need is especially important in responding to inquiries from the Congress and others on subjects not usually associated with dentistry. It also gives us a clue as to the dental research being performed and sponsored outside of the NIDR.

An important fringe benefit of the Agreement is having NLM indexers doing the indexing and revision of dental research abstracts. This arrangement lessens the workload on Institute staff and should result in greater precision in retrieval.

Under the terms of the Agreement, NIDR will be responsible for the collection; indexing; and creation of a machine-readable magnetic tape in standard NLM input format. We plan to do this on a quarterly basis. The NLM will mount the database and make the file available through its MEDLARS system; do necessary testing and Class Maintenance changes to the database; announce the availability of the file in its publications; and provide standard documentation and training. An option remains to convert the tape to an annual publication if the demand for hardcopy is great enough.

FEDLINK

The Interagency Agreement with the Federal Library Information Network through the Library of Congress continues to be an efficient and cost effective link between the Institute and commercial information vendors. At present we provide support to three -- BRS, DIALOG, and PAPERCHASE. In turn, each of these vendors (with the exception of PAPERCHASE which is limited to MEDLINE) give us access to hundreds of databases. There are over 110 such vendors to choose from if the need were justified. As could be expected, the greatest users of this service are the intramural investigators.

Computerized Graphics Inhouse

The RDMIS Model 80 PS/2 personal computer purchased last fiscal year will shortly be equipped with a hardware add-on device that will allow staff members from the OD, EODPP and EP to produce low cost, high quality slides of both text and graphic material. Presently, such graphic material is produced by NIH Graphic Arts at considerable expense both in time and money. In the near future, Institute staff will have the capability of producing slides at about $.50 per slide within the timeframe of an hour. This is by far cheaper and faster than using the NIH Graphic Arts Department, and $7.00 per slide cheaper than producing the material via the Harvard Graphics software package and sending diskettes to a private contractor for slide production. The slides produced for presentation at the last IADR by members of the EODPP staff employed this technology (Harvard Graphics) but relied on the slide production by a contractor. The funding for this hardware was a joint effort of the OD, EODPP and EP which will include purchase, at a later date, of a laser printer for the RDMIS Model 80 thus providing a high quality printing capability.

Personal Computers at NIDR

Due to an ever increasing demand for Personal Computer expertise, the RDMIS has been deeply involved in all areas of microcomputer integration for the Institute. This section has become a focal point for NIDR employees who need assistance in purchasing, installing, using, and troubleshooting Personal Computers and its ever volatile technology. Technical consultation is provided on both hardware and software issues, new computers are "set-up" according to DCRT recommendations, software packages (SAS, Word-Perfect, Harvard Graphics) are evaluated and installed, and upgrades to existing systems are added when new products become available. It is anticipated that within the near future these stand-alone PCs will be networked together in the OD, EODPP and EP providing a more efficient office automation capability.

Local Area Network (LAN)

In line with this, a member of the RDMIS staff has been appointed the NIDR representative to the OD NIH local area network (LAN) described as the Campus Backbone. Duties in this area involve insuring that systems purchased and networks established within the Institute are compatible for future inclusion in the Campus Backbone network. This individual is also working with the OD NIH staff to activate the NIDR as a "node" on this network.

ADP Coordinating Committee

The Section Chief chaired, and several members of the RDMIS staff were actively involved as members of, the NIDR ADP Coordinating Committee. The Committee was charged with developing Guidelines for the Procurement of Office Automation Equipment. The Guidelines, which were developed to assure future connectivity with other networks within the Department were completed and distributed in March.

CRISP Collaboration

The RDMIS, in collaboration with the Division of Research Grants, has been involved in the design and development of a user-friendly, WYLBUR based program to be used in conjunction with DRG´s CRISP System. CRISP, an acronym for Computer Retrieval of Information on Scientific Projects, is a large computerized database containing information on Research Projects funded or supported by the U.S. Public Health Service. In addition to administrative/fiscal data, CRISP contains scientific Abstracts and Indexing Terms used to describe a research project. The new system will allow access to all CRISP files from FY 1972 through FY 1988, and can perform searches based on CRISP Abstracting Term, Investigator´s name, Project Number, BID, Activity Code, Institution, or State/Country. This new program was designed for all individuals, regardless of their computer experience. Computer novices and experts alike who seek various grant information will benefit from this system. Users can easily build, process, and retrieve all search results within minutes.

Employee Related Activities

All members of the RDMIS staff are encouraged to attend classes and obtain training that will improve their job skills and most have chosen to do so. This is especially critical in a field that is changing as rapidly as is computer and information science. We have been cooperating with the Office of Personnel to provide training and job experience to an employee in an effort to expand her opportunities here or elsewhere as a Data Transcriber with very positive results.

One of our staff is chairman of the Telephone Committee and co-chairman of the Reasonable Accomodations/Accessibility Committee for the Handicapped Employees Committee. She also participated in the Annual Meeting of the President´s Committee on Employment of the Handicapped, May 4, 1988. Another one of our staff is the NIDR representative to and secretary of the NIH Extramural Programs ADP Coordinating Committee.

Privacy Act/Freedom of Information Act

All Privacy Act and Freedom of Information Act requests received by the Institute are routed through the Section Chief who logs them into the NIH tracking system and coordinates their response. This year saw a change in the fee schedule and other rules and regulations governing the Freedom of Information Act. The Institute has four Systems of Records under the Privacy Act that had to be reviewed this year and both Acts require an annual report of activities.

FINANCIAL MANAGEMENT OFFICE (FMO)

The Financial Management Office coordinates the Institute´s financial activities for the Office of the Director, including the development of the Institute´s budget and its execution during the fiscal year. The FMO is also the Institute´s repository for accounting and payroll records, statistical data and legislative reports, and serves as principal staff advisor to the Director on all financial matters relating to the Institute´s appropriations.

NIDR Budget: Fiscal Years 1989 and 1990

During FY 1988, the FMO formulated the Institute´s FY 1990 President´s budget request by developing the initial budgetary levels; prepared justifications and statistical materials that supported the request; and kept the Institute Director apprised of changes made through negotiations as the budget was transmitted through channels for incorporation into the President´s budget submission to Congress.

Because of the overlap in the budget cycles, the FY 1990 budget is being negotiated as the FY 1989 budget request is being reviewed by the Congress for legislative enactment. If approved, the FY 1989 request will be incorporated into an appropriation bill. As part of this budget process, the FMO staff accompanied the Director to the formal Congressional Appropriations hearings and provided to the Committee additional justification materials as needed. After action on the request by both houses of Congress, the FMO prepared statements that summarized the effect of the Congressional action on the Institute´s budgetary operations and program goals for the next fiscal year.

While the formulation and legislative processes were in progress, staff monitored all grant, contract, intramural and direct operating expenditures for the current year and provided managerial and financial advice regarding the Institute´s extramural and intramural programs. When necessary, they worked with Institute administrative staff to ensure that reprogramming actions were initiated in areas where additional funds were required. The FMO also apportioned funding by quarter to support planned activities and, at the end of the year, prepared the actual obligation report requirements.

Automation

The FMO has expanded its activities through the use of microprocessors and automated data systems, automating its financial operations and initiating new uses for personal and mainframe computers. The office continues to be an innovator in enhancing productivity through the use of electronic and automated equipment.

Other Activities

The FMO provided special reports and monitored the Institute´s trans-NIH activities, which include, among others, research in diabetes, arthritis, nutrition, disease prevention, and AIDS. Five-year forecasts were prepared by this office for planning purposes. The FMO was also responsible for responding to numerous inquiries from Congress, the Office of Management and Budget, and other Federal and nonfederal agencies regarding NIDR program and financial data.

PERSONNEL MANAGEMENT SECTION (PMS)

The PMS is the focal point for personnel management activities which encompass staffing and placement (including merit promotion), classification and pay management, employee relations, and employee development and training.

Reorganizations

Two major reorganizations took place during the fiscal year. In the Epidemiology and Oral Disease Prevention Program, two new branches were established. The Analytical Studies Branch replaced the Science Transfer and Research Analysis Branch, and the Disease Prevention and Health Promotion Branch replaced the Disease Prevention Branch. The Epidemiology Branch was reordered, establishing three new sections (Periodontal Diseases, Caries and Soft Tissue, Craniofacial Defects and Pain). In the Intramural Research Program, the Laboratory of Microbial Ecology was formed from the Laboratory of Microbiology and Immunology, which was renamed the Laboratory of Immunology. The Laboratory of Oral Biology and Physiology was renamed the Laboratory of Cellular Development and Oncology. In the Bone Research and Clinical Investigations and Patient Care Branches, new units and/or sections were established.

Staffing and Recruitment

Staffing and recruitment activities received considerable attention again this year. Due to the retirement of two key management staffers, the Office of the Director saw recruitment for an Executive Officer and a Budget Officer. In the Intramural Research Program, a Deputy Scientific Director was named.

Other major staffing issues involved the constantly changing policies of the PHS Commissioned Corps, particularly those involving senior officers; the new PHS policy involving licensure for all health care providers; the increasing delegations of authority from the Office of Personnel Management (OPM) for the examination and direct hire for many civil service occupations; and the newly established leave transfer program.

Classification and Pay Administration

The PMS staff carried out its regular classification program. Because of a Departmental directive, the requirement to review each position once every two years was eliminated. In response to the annual request, the PMS'reported on the recruitment and retention of employees in the special rate categories, particularly those clerical/technical staff where typing, stenographic or dictating machine transcribing skills are involved, in anticipation that the OPM will increase the salary levels for these employees.

Employee Relations

Again this year, much attention was placed on the Federal Employees Retirement System (FERS), as the OPM decided to hold a belated Open Season to allow employees another opportunity to transfer to FERS. Amid much confusion concerning the requirements and advantages, the PMS staff again counseled employees, and made retirement calculations and comparisons of the old CSRS and FERS. Additionally, a variety of material was distributed on the additional transfer opportunity, as well as on both Open Seasons for the Thrift Savings Plan.

Awards

The Institute continues to have an active awards program, as its managers and supervisors nominate staff for recognition for both NIDR and other incentive and honor awards. As the result, some 75 staff members were recognized at the Annual NIDR Awards Ceremony in November. Staff were recognized for special acts or service, high quality work performance, and for their special or professional contribution to the mission of the NIDR, NIH, PHS or DHHS.

The PMS staff focused considerable attention on a new Departmental performance award system for EPMS staff. The new system, to be operational in 1989 for performance during 1988, emulates the award system presently in place for the PMRS and SES/SSS staff. PMS staff attended briefings on the new system, developed options for consideration by NIDR Executive Staff, and assisted them in installing operational guidelines for its implementation in NIDR. In September, the PMS held information sessions for managers, supervisors and employees to describe the new system.

EEO Activities

The staff continues to collaborate with the NIDR EEO Manager and the NIDR EEO Advisory Committee on matters of joint concern. They participate in advisory committee meetings to keep the EEO community informed about Institute personnel policies and procedures. They particpate with the EEO Manager in the implementation of new or revised EEO policies, as appropriate. The staff also works closely with the EEO Manager and with program managers to assure the feasibility and legality of personnel activities related to affirmative action and equal opportunity.

Professional Activities

PMS staff continued their participation in trans-NIH personnel activities such as career and job fairs, personnel operations workshops, the qualifications review, interview and selection process for the NIH Career Curricula program, as well as continuing membership in the International Personnel Management Association.

EQUAL EMPLOYMENT OPPORTUNITY PROGRAM (EEO)

Public Law 92-261, the Equal Employment Opportunity Act of 1972, requires that all Federal personnel actions be free from discrimination and that affirmative action programs be developed to carry out the purpose and intent of the Public Law. The National Institute of Dental Research affirmative action and civil rights programs are centered in the Institute´s Equal Employment Opportunity Office. This office serves as the principal source of information for and advisor to the Director and to senior management on matters of equal employment opportunity, affirmative action, Federal Equal Employment Opportunity Recruitment Plan, civil rights, and contract compliance. In addition, the EEO office is responsible for the special emphasis program for Hispanics, monorities, women, and the handicapped.

The EEO Program continues to be involved in numerous activities with minority schools, prepares reports and analyses of the Institute´s profile, and arranges seminars which are designed to increase the awareness of minorities, women, and the handicapped about career opportunities.

Discrimination Complaints

The Institute had two formal discrimination complaints filed in 1988. The EEO Manager and Counselor continue to provide, on an as needed basis, career counseling and guidance on job applications, training opportunities, and problem-solving in supervisor/employee relations.

EEO Advisory Committee

The NIDR EEO Advisory Committee serves as a liaison between NIDR employees and management. Its purpose is to define and make recommendations on Institute employee problems wherever they may exist and to advise the Director and his staff of these concerns. The Committee promotes and seeks to achieve equal opportunity through career development, education and training, and related activities without regard to race, color, religion, sex, age, national origin or handicap. Also serving as members of the Committee are representatives to the NIH Federal Women´s Program, the NIH EEO Counselor, the NIH Handicapped Employees Advisory Committee, and the NIH Hispanic American Advisory Committee.

In November 1987, the EEO Advisory Committees of the NIDR, the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Institute of Neurological and Communicative Disorders and Stroke sponsored an "Extramural Training Opportunities Seminar." The Advisory Committees felt this was an excellent opportunity for NIH employees to become aware of specific training programs and activities that are available on campus. The Director for Extramural Staff Training, NIH, provided an overview of the Extramural Associates Program, the Grants Associates Program, the Health Scientist Administrator Programs, and the Professional Development Assignment Training Program. Approximately 35 scientists attended this training seminar.

Continuing the informational sessions, NIDR´s EEO Advisory Committee in February held a lunchtime seminar on career advancement. NIH training staff were available to provide pertinent information on the Management Intern, the Career Curricular, and the Stride Intern Programs. As a result of the special efforts, two employees submitted applications to the Management Intern Program, one employee (minority male) applied and was accepted to the Stride Intern Program, and two employees (nonminority female and minority male) submitted applications to the Career Curricular Program, one of whom was accepted.

Recognizing the month of March as Women´s History Month, the EEO Advisory committee held a special forum with Dr. Marie Nylen, Associate Director for Extramural Program. The theme for this celebration was "Women Making History in NIDR." Dr. Nylen shared with participants some of her experiences in pursuing her education and research goals as well as important concepts of career development. Approximately 65 NIDR employees attended this very inspiring presentation. In continuing with the celebration honoring women in research, posters were developed recognizing five Institute scientists.

The Committee also nominated to the Director two deserving individuals for the NIH EEO Special Achievement Award. These individuals will be recognized for their invaluable contributions to equal employment opportunity at the Institute´s Awards Ceremony in October 1988.

Each year the NIDR EEO Office sponsors training for the EEO Advisory Committee on the Federal equal employment opportunity laws and regulations which prohibit discrimination. The Handicapped Program and Disabled Veterans Program are highlighted during the training session.

Recruitment and Awareness

The EEO Manager continues to identify and communicate with minority, handicapped, and women´s organizations and associations concerning our mission and activities. This office, in conjunction with NIDR Handicapped Advisory Committee representatives, participated in the National Symposium on Perspectives on Employment of Persons with Disabilities and the Annual Meeting of the President´s Committee on Employment of the Handicapped for networking and for discussing dental research.

NIDR each year provides reasonable accommodation for disabled employees during inclement weather. This past year three employees established agreements under the "Inclement Weather Policy."

To increase the awareness of Institute employees about sexual harassment, the EEO Manager designed a special "NIDR EEO Bulletin on Sexual Harassment" for distribution to all employees. The Manager also participated in a special workshop on awareness and prevention of sexual harassment sponsored by the Division of Equal Opportunity.

Staff participated in the 45th Joint Annual Meeting of the National Institute of Science Beta Kappa Chi Scientific Honor Society and the Brookhaven Semester Program in Chapel Hill, North Carolina. Approximately 400 science students participated in this meeting. NIH staff provided a forum for the participants on certain NIH programs and a session on "Marketing Yourself: The Importance of a SF-171."

The EEO Manager represented NIDR at a Symposium on Career Opportunities in Biomedical Sciences in Atlanta, Georgia. The symposium was sponsored by Morehouse Medical College and the Association of Minority Health Professional Schools. These groups are attempting to help reverse the trends of underrepresentation of minorities in the biomedical sciences.

During the past fiscal year, the EEO Office sponsored an informational seminar and several training courses for NIDR employees. As a result of the excellent response to the Sign Language Course begun at the end of 1987, training continued for another eight weeks, crossing into the current fiscal year. To assist NIDR support staff in overcoming resistance to new technology, two courses in office automation--Introduction to Microcomputers for Support Staff and Introduction to WordPerfect--were planned specifically for the NIDR. A videotaped presentation on "Image and Self-Projection for Today´s Professional Women" also was arranged for Institute employees.

In cooperation with other NIH EEO Offices, the NIDR EEO Manager conducted four tours of the Institute´s research facilities for groups of minority, women, and handicapped students.

Affirmative Employment Program (AEP) for Minorities and Women

NIH received new instructions for the development and submission of Federal Affirmative Employment Multi-year Program Plans, Annual Accomplishment Reports, and Annual Plan Updates for FY 1988 through FY 1992. BIDs with a staff of 200 or more employees (including NIDR) were required to develop affirmative employment initiatives that cover the full multi-year affirmative employment planning cycle. The AEP was based on a work force profile, an underrepresentation analysis, and the identification of objectives and action items.

Minority Biomedical Research Support Program
Minority Access to Research Careers Program

Through cooperative agreements with the National Institute of General Medical Sciences and the Division of Research Resources, the NIDR supports components of the Minority Biomedical Research Support (MBRS) and Minority Access to Research Careers (MARC) Programs that relate to the overall mission of the Institute. In 1988, the EEO Manager and five NIDR scientists participated in the NIH Centennial MBRS-MARC Symposium held in Washington, DC. This symposium provided an opportunity for staff to discuss research training opportunities at NIDR with faculty and students.

Community Outreach

Two employees from the Intramural Program represented the NIDR as special judges in the District of Columbia Annual Science Fair. The group reviewed 16 dental-related science projects. Two deserving students were recognized for their outstanding efforts. Each student received an award, a book, and several publications on the mission of NIDR.

The EEO Office continues to provide Ballou Senior High School, Morehouse College, Meharry Medical College School of Dentistry, and the University of Puerto Rico School of Dentistry with the Institute´s surplus scientific books, journals and slides. This service has now been expanded to include two historically Black colleges: Tougaloo College in Tougaloo, Mississippi and North Carolina Agricultural and Technical State University, Greensboro, North Carolina. Since the inception of the Resource Collection program, approximately 190 journals have been identified for distribution and over 425 books have been contributed to the project.

Civil Rights

The EEO Manager continues to serve as the Institute´s Federal Contract Compliance Coordinator. New contracting and project officers in the Institute completed training on contract compliance and administered the EEO Check List for nonconstruction contracts in accordance with Executive Order 11246. The Institute continues to participate in the NIH Consultant File on Committees/Advisory Groups, the NIH Visiting Professor Program, the Small and Disadvantaged Business Program, and the Small Grants Program.

EXTRAMURAL PROGRAM

ANNUAL REPORT - FY 1988

REPORT OF THE DIRECTOR

EXTRAMURAL PROGRAM, NIDR

The Extramural Program of the National Institute of Dental Research (NIDR) is responsible for the development, review, funding, and management of grants seeking Institute support for research, research training, and manpower development. Three program branches support a wide spectrum of research that extends from basic research to new methods of diagnosis, treatment and prevention. A special assistant is in charge of training and manpower development programs, while review and grants management are the responsibility of a Special Review Branch (SRB) and a Grants Management Section respectively.

A broad array of award mechanisms is available to extramural scientists ranging from small feasibility grants to regular grants to large center grants. During FY 1988, the NIDR Extramural Program (EP) made 633 research and training awards for an estimated total of $ 90 million including 401 investigator-initiated research project grants ($61 million). The 401 research project grants accounting for 67% of the EP budget comprised 325 regular grants for $45 million; 13 program project grants for $7.5 million; 11 young investigator awards for $.6 million; 29 FIRST awards for $2.8; 15 MERIT awards for $3 million; and 8 small business innovative research awards for $1.6 million, an increase of $ 400,000 over the funds specifically set aside for that purpose. Of the 401 research project grants, 60 were new awards and 60 were renewals of existing awards.

The NIDR also funded 16 center grants for an estimated total of $14 million or almost 16% of the EP budget. The center awards supported five multi-categorical centers, one Oral Health in Aging Center, five Periodontal Diseases Research Centers, three Caries Research Centers, and two Pain Research Centers. Four awards were new awards and another four represented renewals of existing awards. The multi-categorical institutes receiving support in FY 1988 included one of the original Dental Research Institutes and Centers. First funded in 1967, it entered its 22nd and final year of funding, bringing to a close an important and successful phase in the history of NIDR support for research centers.

The "Other Research" budget, approximately 10% of the EP budget, funded 31 small grants for $.7 million and 16 small instrumentation grants for $287,219. It also provided $ 152,611 as full or partial support for 10 conferences and $159,036 for the Division of Research Resources Minority Biomedical Research Program. Eighty percent of the OR budget or $7.5 million funded

17 career development awards, 22 individual physician scientist for dentist awards, 22 individual dentist scientist awards and 9 institutional dentist scientist awards, all together providing support for a total of 124 individuals.

The $5,4 million National Research Service Award (NRSA) budget was disbursed as follows: $4 million in support of 27 regular institutional training programs and 23 institutional awards to provide short-term (summer) training opportunities for dental students; $1 million for 43 individual fellows; and $109,443 for meeting-related training activities. The 27 regular institutional training grants provided 15 predoctoral and 117 postdoctoral training positions while the short-term awards supported a total of 148 dental students.

Special Emphasis Areas: AIDS: NIDR extramural support for AIDS-related research reached $2 million, an increase over the previous fiscal year of $ .7 million and almost twice the amount budgeted for this activity. Included in the portfolio were 2 program project grants, 6 regular research grants, 1 FIRST award, and 3 career awards.

Minority Programs: The NIDR provided $223,046 in direct grant support (1 regular research grant and 3 small grants) to minority institutions. In addition, the NIDR funded 2 minority research supplement awards totalling $112,052. The contribution to the Minority Biomedical Research Support Program administered by the Division of Research Resources was $159,036, a 36% increase over the FY 1988 budget figure. These funds supported dental research projects at four different institutions. In addition, $26,960 was provided the Minority Access to Research Careers program administered by the National Institute of General Medical Science in support of four ancillary training activities. Funding ($10,065) was also provided for a new short-term training program intended only for minority dental students.

EP activities were varied and extensive. The most demanding and time-consuming efforts of the year involved review by the SRB of eight applications for Periodontal Diseases Research Centers support and of 14 applications for Centers on Oral Health in Aging support. Both reviews utilized the "reverse site visit" format. However, in the case of the Aging Centers, the reverse site visit was preceded by a "triage" review which reduced the number of competitive applications from 14 to 7. Five Periodontal Diseases Research Centers were funded representing one first time application and four competing renewal applications. Only one Center on Oral Health in Aging was funded. However, most of the unsuccessful applicants are expected to submit amended regular grant applications for the October 1, 1988 deadline.

Other major activities were the development of requests for applications (RFA) for Caries Research Centers, Craniofacial Anomalies Research Centers, Materials Science Research Centers, Dentist Scientist Award - Institutional Grants, and National Research Service Award - Institutional grants. Three Caries Research Centers were established in FY 1984. With the RFA issued this year, the NIDR, with Council endorsement, has indicated its intent to continue this program while at the same time opening it up for competition by other institutions. By the September 1 deadline, five applications were received in response to the Caries Research Centers RFA including applications from the three existing centers and two new institutions. The two other center RFAs represent new initiatives with submission deadlines in FY 89.

EP staff visited a number of grantee institutions as part of a continuing effort to keep the scientific community informed of institute programs. Staff also participated in numerous site visits and attended scientific meetings using these occasions as opportunities for further contact with the scientific community. In addition, EP staff played a major role in the planning, organization and conduct of the following conferences: Molecular and Genetic Basis of Growth and Development, held at NIH February 22-23, 1988; Prenatal Craniofacial Development, held at the National Institute of Environmental Health Science April 20-22, 1988; Consensus Conference on Dental Implants, held at NIH June 12-15, 1988. Four other conferences held during the fiscal year (Multidisciplinary Management of Cleft Lip and Palate; Biological Mechanisms of Tooth Eruption; Bioactive Factors in Bone Development and Repair and World Workshop in Oral Medicine) received conference grant support from the NIDR.

The success of our efforts are dependent on the existence of a strong partnership between the extramural community and the NIDR. The willingness of the extramural scientific community to serve on peer review committees and advisory committees is essential to the success of our many initiatives and last, but not least, their own research is the force that moves science forward. This report reflects some, but by no means all, of the progress achieved during the past year.

ANNUAL REPORT - FY 1988

RESEARCH TRAINING AND MANPOWER DEVELOPMENT

EXTRAMURAL PROGRAM - NIDR

We presented to the National Advisory Dental Research Council (NADRC) at its January meeting, our plan for implementing the thirteen recommendations made by the adhoc "Consultant Panel" to our NRSA program. The recommendations resulted in new policies for the administration of our NRSA program and were incorporated into a Request for Applications (RFA) which appeared in the July 15, 1988, issue of the NIH Guide for Grants and Contracts. The recommendations and new policies are summarized as follows:

1. A single annual NRSA training grant receipt date and review cycle for all new and competitive renewal applications was initiated with this RFA. This new schedule is designed to allow Program Directors time to recruit candidates during the fall of an academic year for appointments to begin the following summer.

2. Each year the RFA will indicate the areas in which the Institute wishes to receive applications. The current RFA announced our interest in applications proposing training in the following: molecular and cellular biology as related to studies of mechanisms underlying development, function, disease, and repair of oral tissues; nutrition; epidemiology; and neurobiological/neuroanatomical aspects of oral motor function and orofacial pain.

3. Training grants should propose programs in which the training is part of a research degree program. Institute priority will be given to proposals that offer dentists the opportunity of obtaining a Ph.D. degree; in certain cases, proposals offering training as part of a masters degree program will be acceptable.

4. Dentists requiring additional time to complete training as a participant in a Ph.D. program may anticipate favorable consideration of a waiver request, contingent upon certification of the recipient's good academic standing.

5. There will be no upper limit to the number of postdoctoral or predoctoral trainee positions an applicant may request. However, training programs with fewer than five positions for postdoctoral trainees over the five year period will not be funded.

6. Only one training grant will be accepted from an institution, unless distinctly different training programs are proposed.

7. Individuals with a research doctoral degree (Ph.D. or equivalent) are expected to apply for the individual postdoctoral fellowship award (F32). As a rule, they should not be appointed to the traditional institutional training grant (T32).

The August 14, 1987 issue of the NIH Guide for Grants & Contracts, announced the availability of NRSA "Institutional Research Training Grants in Primary Medical & Dental Care". The NIDR was one of the three participating NIH Institutes, in addition to the National Institute of Mental Health. We reviewed eleven applications and funded one, which began on July 1, 1988.

The NIDR announced the availability of the Dentist Scientist Award in the June 29, 1984 issue of the NIH Guide for Grants and Contracts. The initial application receipt date was October 1, 1984, and the first awards were made in the summer of 1985. Therefore, those first institutional Dentist Scientist Awards (K16s) will terminate their first competitive five year award periods in June 1990. In order to allow the K16 program directors sufficient time to recruit and manage their programs, the NIDR issued an RFA for new and renewal institutional DSA (K16) proposals. We anticipate that all nine of the currently active K16 programs will submit renewal applications. In addition, we expect at least seven new applications which will be reviewed in competition with the renewals.

The third meeting of the program directors of the institutional Dentist Scientist Award (K16) took place in March, 1988, in conjunction with the IADR/AADR meeting in Montreal. Issues important to the efficient operation of the K16 program were discussed, such as, funds for supplies and equipment, minority participation, recruitment and the applicant pool, candidate application format, and starting salaries. The program directors again stated that this meeting was very useful and should continue to be held, at least once a year.

The Physician Scientist Award for Dentists (K11) and the Dentist Scientist Awards (K15-individual and K16-institutional) continue to receive considerable interest in congress and the academic community. As a result, we were able to fund three new K11, and four new K15 awards this year. In addition, the nine institutional awards were each allowed to add two more additional "clinical research scholars" (CRSs) to their programs. Therefore, the NIDR is currently supporting approximately 107 CRSs at some stage of their research/clinical development.

At the conclusion of this fiscal year, the NIDR will be supporting approximately 266 trainees, fellows, and clinical research scholars, at some stage of their research/clinical training. Of these, approximately $5.7 million are in the Periodontal and Soft Tissue Diseases Research Branch, $3.6 million in the Craniofacial Anomalies, Pain Control, and Behavioral Research Branch, and $3.5 million in the Caries, Nutrition, Salivary Sciences, and Restorative Materials Research Branch.

ANNUAL REPORT - FY 1988

CARIES AND RESTORATIVE MATERIALS RESEARCH BRANCH

Extramural Program, NIDR

Progress in dental caries research includes: epidemiological studies of the incidence and prevalence of root surface caries in special populations, and the prevalence of caries and fluorosis in children; studies on the mechanisms of bacterial attachment in supragingival plaque; the application of molecular genetic approaches in the isolation of S. mutans genes involved in the virulence and sucrose utilization; studies on the role of the mucosal immune system in caries prevention; and a variety of approaches to define the etiology, pathogenesis and prevention of dental caries. Progress in restorative materials research included studies to improve the wear resistance and color stability of composite resin materials; a recent development in adhesive bonding technology; studies to improve porcelain fused to metal prosthetic materials, and studies to assess the clinical performance of a castable ceramic material. Recent progress in research on salivary glands and their secretions included studies on the developmental and hormonal regulation of epidermal growth factor gene expression in the mouse submandibular gland, tooth surface-pellicle lipids and their role in the protection of dental enamel against lactic acid diffusion in man, differences with caries regarding the susceptibility of salivary mucin to proteolysis, biochemical and biophysical properties of two mucins from human submandibular-sublingual saliva, structure-activity relationships in the inhibition of calcium phosphate precipitation by human salivary acidic proline-rich proteins, lubrication and viscosity features of human saliva and commercially available saliva substitutes, lipid composition of parotid saliva in Sjögren's syndrome in man, and concentration of α-amylase and secretory IgA in stimulated parotid saliva from people of various ages.

A01 Determine the incidence and prevalence of dental caries among all target populations.

Epidemiologists are conducting studies to determine the prevalence and incidence of root caries and coronal caries in elderly and compromised groups. They have identified associations between root surface caries and a variety of clinical, laboratory, dietary, and psychosocial correlates.

A representative sample of 338 dentate rural Iowans age 65 and older were examined at baseline, 18 months, and 36 months. At baseline, the participants averaged 18.8 teeth remaining, 14.53 decayed and filled coronal surfaces and 2.35 decayed and filled root surfaces. Gingival recession was present on an average of 29.8 root surfaces. These were the surfaces considered at risk for root caries. Over three years, the participants developed caries on an average of 2.4 coronal surfaces and 1.1 root surfaces, indicating that both coronal and root caries were active in this population. There were no differences in increments between men and women. People who lived in nonfluoridated areas for 30 years or more had a greater risk of coronal and root caries. The best predictor of tooth loss in the study population was previous coronal and root caries.

Another study was carried out to determine the prevalence of root caries in an older adult population resident in the city of Birmingham, Alabama. Subjects (603) were at least 60 years of age and had at least 15 natural teeth. The mean number of teeth (±SD) was 23.5 ± 4.0, and the mean RCI rate was 8.1 ± 9.5%. Of the subjects, 69.7% had root caries and 100% had gingival recession. The mandibular second molars had the highest rate (28%) and the mandibular central incisors the lowest (2%). The rates were significantly higher in males than in females and in whites than in blacks.

As part of a collaborative, multidisciplinary investigation of root surface caries (RSC), carried out by scientists at Forsyth Dental Center, 223 volunteer subjects (median age = 57) were screened for coronal caries, debris, calculus, gingivitis, periodontal pocketing, abrasion, recession, as well as RSC. The entire study group was divided into three subgroups: 1) subjects without root surface lesions or restorations (non-diseased individuals; (2) subjects with one or more root surface lesions (diseased individuals); (3) subjects with one or more root restorations but no lesions. Distinctly different clinical profiles for non-diseased subjects (subgroup 1) and diseased subjects (subgroup 2) were observed. Subjects without RSC had more teeth, fewer diseased, missing, or filled teeth, fewer crowns, less recession, less debris, less calculus, and less gingivitis. On the other hand, diseased and non-diseased subjects did not differ with respect to abrasion and periodontal pocketing. Results for subgroup 3 fell between those of 1 and 2. The data indicates that individuals without RSC may be distinguished from those with the disease on the basis of a variety of measurable clinical features.

Dental scientists, also at Forsyth, have assessed the level of root surface caries in a population of diabetic adults. Diabetics are considered to be a group of special interest because they are alleged to be periodontally compromised and have atypical patterns of refined carbohydrate ingestion. Eligible subjects had to be between the ages of 45 and 65, have a minimum of 10 teeth, and 3 sites with gingival recession. They were examined for coronal caries, oral hygiene, gingivitis, pocket depth, recession, and root caries. Data from these individuals was compared to data from selected matched non-diabetic controls. The groups were found to be similar with respect to diseased, missing and filled teeth, extent of gingival recession, number of carious root lesions, and most of the other parameters. There was a distinct difference, however, with respect to restored root surfaces; 1.8 mean filled surfaces in the controls as compared with 0.5 in the diabetics. Root restorations can be attributed to either prior carious lesions or abrasion. The authors indicate that there is no apparent reason why diabetics should have less abrasion, and suggest that their reduction in restored root surfaces is the result of a restricted ingestion of refined carbohydrates.

A02 Conduct periodic surveys on the prevalence of dental fluorosis.

Epidemiologists have related the prevalence of caries and fluorosis among Michigan children to the various concentrations of F in four communities' water supplies. Children ages six to 12 were screened for caries and for fluorosis of the permanent dentition. Approximately 65% of all children were caries-free, ranging from 55.1% in fluoride-deficient community (0.0 ppm) to 73.7% in the community with 1.0 ppm F. The prevalence of caries was significantly associated with age, dental attendance, and the use of a water supply fluoridated at 1.0 ppm. About 36% of all children had dental fluorosis, ranging from 12.2 in the lowest F area to 51.2 in the community with 1.2 ppmF. All of the fluorosis was very mild. Results suggest that children in the four communities may be ingesting a similar level of F from sources such as dentifrices, dietary supplements, and professional applications, but the factor that differentiates them with respect to the prevalence of caries and fluorosis is the F concentration in the community water supply.

A04 Further characterize the composition and chemistry of plaque, tooth enamel and physiological systems.

The oral streptococci are important members of the microflora of dental plaque and polysaccharides produced by them from sucrose make an important contribution to the formation and metabolism of plaque. These glucans and fructans are synthesized by the microbial extracellular enzymes glucosyltransferase (GTF) and fructosyltransferase (FTF), respectively. The synthesis of adherent water-insoluble glucan is involved in the colonization of mutans streptococci in plaque and depends on simultaneous activity of two enzymes, GTF-I and GTF-S. Recently, several laboratories have introduced a genetic approach to the study of GTF and have used recombinant DNA techniques to clone the respective genes into *E. coli*. The complete nucleotide sequences of two genes gtfI gene from *S. sobrinis* and gtfB gene from *S. mutans* have been determined; both genes encode a GTF which makes insoluble glucan. The availability of these sequences allowed for comparison at the molecular level of the two genes and their deduced proteins. Such an analysis is important because of the involvement of these two species in dental caries and their evolutionary differences.

NIDR-supported scientists are making substantial progress in elucidating the physiological mechanisms involved in acid/base balance, energy metabolism, sugar transport into cells and in understanding other microbial physiological systems involved in supragingival plaque formation, the pathogenesis of caries and disease prevention. Many of these investigators are utilizing genetically engineered or conventionally produced bacterial mutants and/or monoclonal antibodies in their research.

A06 Elucidate the systems allowing bacteria to recognize and attach to different surfaces.

Several scientists are studying interactions between different species of bacteria and between bacteria and saliva-coated enamel surfaces to determine the mechanisms of bacterial attachment in supragingival plaque.

Investigators at the University of Florida, Gainesville, have shown interactions (coaggregations) involving mutans streptococci and actinomyces species not previously reported. The investigators suggest possible ecological relationships which may provide the mutans streptococci with niches in the oral cavity that result in specific patterns of disease such as root caries. This latter disease has an etiology related to both *S. mutans* and the actinomyces under study.

Haemophilus parainfluenzae and *Streptococcus sanguis* are both predominent in developing supragingival plaque. Investigators at the University of Minnesota have reported that coaggregations between these microorganisms appear to involve a glycoprotein molecule on the *H. parainfluenzae surface*. These scientists have also developed an *in vivo* model assay that measured the interbacterial adherence of these bacterial species on a bovine enamel chip placed in the oral cavity. The chip contained a monolayer of *S. sanguis* cells. Streptomycin labeled *H. parainfluenzae* preparations formed coaggregates with the *S. sanguis* cells on the chip *in vivo*. This assay is also being used to study interbacterial adherence of different species of bacteria in situ.

The attachment of strains of *Streptococcus sanguis*, *Bacteriodes gingivitis*, *Actinomyces viscosus* and

Streptococcus mutans to salivary proline-rich-protein adsorbed on hydroxyapatite surfaces has been reported by scientists at Forsyth Dental Center in Boston. These investigators suggest that the adsorbed proline-rich-proteins contribute to *in vivo* pellicle receptors for these bacteria. With respect to *S. mutans*, proteins derived from human submandibular-subgingival saliva, with estimated molecular weights between 300,000 and 320,000, also promoted adhesion to hydroxyapatite. The investigators believe that this salivary component may play a significant role in the initial adhesion of *S. mutans* cells to the surface of dental enamel.

Results from several studies have shown that cariogenic streptococci produce several glucan-binding proteins and have the capacity to bind to glucan-coated surfaces. The recent demonstration of cell-free bacterial glucosyltransferase (GTF) in human whole saliva and in the initial pellicle which forms on the teeth, suggests that glucans present in pellicle may represent binding sites for the selective adherence of oral bacteria. Scientists at the University of Rochester found that both *S. sobrinus* and *S. mutans* adhered more efficiently to hydroxyapatite (HA) surfaces which had been exposed to GTF and then incubated with sucrose, than to HA without associated glucan synthesis. This effect was also observed for glucans formed on HA treated parotid saliva. Glucan formation was critical for the increased bacterial adherence observed. These results suggest that the formation of glucans on tooth surfaces by GTF in the pellicle promotes the adherence of glucan-binding cariogenic bacteria.

A08 Develop and test new fluoride agents and vehicles and apply existing fluoride regimens to new target populations.

Radiation caries is a ravaging type of caries that occurs in patients who receive radiotherapy for head and neck cancers which results in a pronounced and long-lasting xerostomia. A few studies have reported encouraging results with the frequent use of fluoride gels or fluoride-chlorohexidine topical applications plus sodium fluoride mouthrinses to prevent radiation caries in these patients. However, because of the extent of treatment, patient compliance is poor.

In a recent, small clinical trial with xerostomic subjects, investigators at Eastman Dental Center used a twice daily oral rinse containing sodium fluoride (NaF). No new carious lesions were noted in these subjects despite the presence of severely reduced salivary flow. The clearance of fluoride in these subjects, was delayed, resulting in salivary fluoride concentrations adequate to prevent demineralization and caries development. The lack of caries development in the subjects using the NaF mouthrinse would indicate that this regimen is as effective against caries as a NaF gel applied in custom trays, and its ease of use should result in better patient compliance, an extremely important issue in these subjects on long-term fluoride therapy.

A-12 Purify and characterize all potentially _S. mutans_ antigens for vaccine development.

Immunologists at the University of Alabama have found evidence for a common mucosal immune system in humans by demonstrating salivary antibodies after peroral administration of _S. mutans_ antigens. In their study, peripheral blood mononuclear cells from human volunteers who had ingested capsules containing killed _Streptococcus mutans_ were assayed for spontaneous antibody-producing cells. Volunteers exhibited circulating IgA-producing cells within 7 days. One IgA-deficient subject exhibited IgM responses. Significant S-IgA anti _S. mutans_ antibodies were detected in saliva and tears by day 14. No changes in serum anti-_S. mutans_ antibodies were noted. The IgA-deficient subject produced salivary secretory IgM antibodies. These results suggest that, after antigen ingestion the sensitized gut-associated lymphoid cells migrate via peripheral blood as antigen-specific precursors of IgA and that their presence precedes the appearance of S-IgA antibodies in external secretions such as saliva. Further studies are being carried out with purified _S. mutans_ surface antigens to measure antibody formation and effects on _S. mutans_ colonization.

Other investigators at Forsyth Dental Center have studied the effect of oral administration of glucosyltransferase (GTF) from _Streptococcus sobrinus_ 6715 on levels of immunoglobulin A (IgA) antibody to GTF in parotid saliva and on the number of indigenous _Streptococcus mutans_ in the whole saliva. Two regimens of immunization were given, the second 3 months after the first. After the first immunization regimen, the GTF vaccine group exhibited higher parotid saliva IgA antibody elevations than a placebo group. Between the first and second immunization regimens a significant increase in parotid salivary anti-GTF activity also occurred in the GTF vaccine but not the placebo group. No significant differences between the two groups were observed for serum IgG or IgA antibody to GTF. These data indicate that oral administration of GTF from the mutans group streptococci has the potential to elicit a salivary IgA antibody response. A consistent effect associated with immunization was retarded reaccumulation of indigenous _S. mutans_ following dental prophylaxis.

Scientists have also shown that gnotobiotic rats could be protected against dental caries by passive immunization with bovine milk antibodies to _Streptococcus mutans_. A multivalent vaccine consisting of whole cell antigens of mutans streptococci was used to hyperimmunize a group of cows. Serum samples from these animals contained immunoglobulin G1 (IgG1) antibody activity to mutans streptococci. Whey obtained from the animal with the highest serum antibody activity, which also contained high levels of IgG1 antibody, was used in passive caries immunity studies. Gnotobiotic rats monoinfected with Streptococcus mutans or Streptococcus sobrinus and provided a caries-promoting diet containing immune whey had lower plaque scores, numbers of streptococci in plaque, and degree of caries activity than similarly infected animals given a diet containing control whey obtained from nonimmunized cows. The active component in the immune whey was the IgG1 anti-S. mutans antibody.

A17 Identify ways to reduce the caries-producing potential of dietary items.

Scientists at the University of Iowa evaluated the acidogenic potential of a well-balanced meal and assessed the effect of chewing gum after the meal. The acidogenic potential of the meal was determined using indwelling wire telemetric appliances containing glass pH microelectrodes. Plaque pH responses were continuously recorded for 120 minutes after a 20 minute meal with and without sorbitol chewing gum in each recording session. The meal resulted in plaque responses below pH 4.5 which did not recover to resting values in the next 120 minute period. Gum chewing after a meal resulted in complete reversal to the resting pH. The pH values did not drop below pH 5.7 during the remainder of the recording session (100 minutes). The investigators believe that neutralization of interproximal plaque acids by gum chewing is the result of stimulation of salivary flow and mechanical "pumping" of saliva to the interproximal sites.

Investigators at the University of Iowa also determined the effect of consuming 5 gms of aged cheddar cheese, 4 times daily on progression or reversal of lesions created in dialyzed acidified acid gel at pH 4.0. Thin sections of lesions were created and examined using polarized light microscopy and microradiography. Sections were placed at proximal surfaces in intra-oral appliances and worn by human volunteers for 2 weeks. The sections were then re-evaluated for changes in mineral content. Human plaque pH changes were assessed using wire telemetry and glass pH microelectrodes. Sucrose control rinses were used to establish acidogenicity prior to consumption of the cheese. Sucrose challenges were used after cheese consumption to determine the effect of the cheese on reduction of the plaque acid challenge. In all cases of cheese consumption, remineralization was seen in both root and enamel lesions. None of the plaque responses to the subsequent sucrose challenge dropped below pH 5.70. Aged cheddar cheese eaten 4 times daily for 2 weeks appears to facilitate remineralization and to act to reduce the acid challenge.

P05 Develop better wear and color stability properties for known and newly developed composite formulations.

The controversy over the use of dental amalgam has focused attention on the replacement of amalgam by posterior composite resin restorative materials. A general change to composite resin for the restoration of posterior teeth would be a major revolution in dental treatment, since silver-tin amalgam has been used routinely for over a hundred years. Thus, the clinical performance and potential of the posterior composite resin restorative materials deserve careful study using scientifically based research methodology. Investigators at the University of Michigan compared the wear resistance of nine composite resins in the complete dentures of twelve edentulous patients at three and nine months. The data were analyzed for location of the restorations, material used, and the patient. The results showed the patient as a significant influence on wear. Neither material nor location of restoration was a significant influence. However, some interactions between patient and material, patient and location of restoration, and material and location of restoration did exist.

Although the color stability of the presently employed dental resins is quite satisfactory, some discoloration of these materials can be observed clinically. Under oral conditions, these restorations are exposed to the combined effects of light, moisture, stains and mechanical wear resulting in visibly detectable and aesthetically undesirable color changes. Many of these changes are the result of photochemical reactions of ingredients in the composite caused by exposure of the restoration to various energy sources. Since clinical studies to determine color stability of restoratives are time-consuming, accelerated aging tests have been suggested to correlate laboratory findings and clinical performance. The color stability of chemically and light cured hybrid and microfilled resins and a material copolymerized by light and chemical aftercure was studied by investigators at the National Bureau of Standards in Gaithersburg. These resins were exposed to irradiation and elevated temperature at 60°C in air or water and under different experimental conditions for various time periods. Results indicated that the color changes of the composites intensified on increasing the irradiation time. Exposure to two light sources under similar conditions gave comparable results. Visible light cured materials were more color stable than two common chemically cured composites. Light shades yielded more visible color changes than dark shades of the same brand. There was significantly more severe discoloration of composites kept in water at 60°C than for those stored in air. Thermal exposure studies of the chemical or light cured, hybrid or microfilled composites aged for 34 months at 37°C in the dark showed no color changes when compared to specimens kept at room temperature. Only the two chemically-cured commercial composites discolored at 60°C within six months in the dark. Even after 2 1/2 year storage at this temperature most of the visible light cured brands showed no, or barely perceptible, color changes. Most water-stored specimens discolored much more rapidly; some within one month and nearly all within six months. All materials gave perceptible or very perceptible color changes on 12 month storage in this medium, and further discoloration was observed after two years.

P07 Study adhesive bonding between tooth structures and composites.

Researchers at the American Dental Association Paffenbarger Research Center in Gaithersburg have perfected a new two-step adhesive bonding system based on the multi-step bonding technology developed in 1981. This adhesive bonding procedure makes the filling adhere to the tooth, making it unnecessary to drill out the healthy tooth material to hold the filling or to use an anesthetic. The first step is to apply an aqueous solution - acidic N- phenylglycine - for 30 to 60 seconds to etch the enamel. After the surface is blown dry, the area is painted with a solution of PMDM (the reaction product of pyromellitic dianhydride and 2-hydroxyethyl methacrylate) and allowed to dry for 10 to 15 seconds. The second step is to apply the composite restorative material to the prepared surface. The main advantage is that the new system allows dentists to treat both dentin and enamel with the same solution simultaneously. Old methods require a delicate process of separate etching of enamel and treatment of dentin involving at least six steps. Also, the new system is easier for dentists to apply, reduces the amount of tooth structure that must be cut away to retain restorative composites and increases patient comfort during and after dental procedures. This new bonding technology has resulted from long term research supported by the NIDR.

P08 Elucidate the role of filler particles in the mechanical and chemical behavior of composites.

Dental composite resins offer a convenient and aesthetic alternative to dental amalgam and metal castings for the restoration of anterior and posterior teeth. However, dental composite restorations undergo physical and chemical degradation in vivo. This reduction in properties for aged composites is believed to be dependent upon the filler composition, as well as the degree of cure of the resin matrix. A laboratory study to determine the effects of filler type and size and aging in solvents on the fracture toughness and yield strength of experimental dental composites was conducted at Baylor College of Dentistry, Dallas, Texas. The results suggest that a degradation of the resin matrix, essentially independent of filler composition, may cause a reduced fracture resistance and yield strength in aged composites.

P15 Develop new and/or improved intraoral prosthetic materials.

Superalloy research has revealed that the adherence of an oxide to its alloy substrate is affected by whether it grows by outward cation diffusion or inward anion diffusion, with inward anion diffusion producing the best oxide adherence. Researchers at the University of Georgia, in collaboration with the Ohio State University, have conducted double oxidation studies to determine the growth directions of oxides that form on dental porcelain fused to metal alloys and to ascertain whether any correlation exists between the oxide growth direction and oxide adherence. The double oxidation technique involved the oxidation of alloy specimens first in oxygen-16 and then in oxygen-18. The relative positions of the oxygen-16 and oxygen-18 isotopes within the oxide layer were determined through depth profiling with surface ion mass spectroscopy (SIMS). The growth directions were determined from the relative positions of the isotopes in the oxide layer. The two alloys studied that formed the most adherent oxides exhibited an oxygen-18 enriched zone adjacent to the oxide metal interface, an indication that at least partial inward growth by short-circuit diffusion had occurred. The two alloys that formed the least adherent oxides exhibited maximum oxygen-18 enrichment at the gas-oxide interface, an indication of oxide growth by outward cation diffusion. Hence, dental alloys, although they are oxidized for much shorter times than those encountered by most superalloys, appear to exhibit the same relationship between oxide growth direction and oxide adherence.

A clinical study to assess the performance of a castable ceramic material intended for use in dental fixed prosthodontics and to determine the operational parameters required for successful clinical function was carried out at the U.S. Army Medical Research and Development Command in Presidio of San Francisco, California. A total of 106 test and control crowns (PFM) were placed in 71 patients and their performance was evaluated over a three-year period. Criteria were developed to assess marginal adaptation, esthetics, surface character of crown, tissue compatibility, and opposing dentition by an ordinal rating system. In addition, replicas of the surface which included a fiducial reference mark were produced for SEM examination of the performance of the overlying shading porcelain. Analysis of crown dimensions and the occlusion were also performed. Analysis of the failures revealed that control of crown thickness and occlusion were of paramount importance to clinical success. The controls remained unchanged. When these factors were carefully controlled in a subsequent study, it was shown that the material was durable, extremely esthetic, possessed excellent marginal fit, biocompatibility and there was no degradation of the overlying shading porcelain.

H01 Correlate the structure of salivary gland cell types with function, in different species and over the life cycle of the glands.

Although the polypeptide mitogen, epidermal growth factor (EGF), has widespread biological effects on the growth and differentiation of various cell types and tissues, on the expression of specific genes, and on gastric acid secretion, its precise physiological roles remain unknown. The regulation of EGF gene expression is as interesting and diverse as are its biological activities. EGF levels vary during development and in response to several hormones. The recent cloning and sequencing of EGF cDNA revealed that the mRNA encoding this small growth factor (53 amino acids) has an unexpectedly long open reading frame, sufficient to encode a 1217-amino acid EGF precursor. The availability of these nucleic acid probes now permits detailed studies of EGF gene regulation to be performed. EGF was first isolated from the submandibular gland of the male mouse, where it is found specifically in the cells of the granular convoluted tubules (GCT). The GCTs are more prominent in the male than in the female, imparting the characteristic sexual dimorphism observed in the gland. The differentiation of GCT cells is postnatal, coinciding with the onset of puberty. The level of EGF in the gland parallels the differentiation of GCT cells; EGF is absent from the gland before 2-3 weeks of age and rapidly increases after puberty. It is about an order of magnitude higher in adult males than in females and declines with senescence in the male. At least three hormones control the differentiation and maintenance of differentiated functions of GCT cells and EGF levels in the submandibular gland. Androgens are responsible for the sexual dimorphism of the gland and for the high levels of EGF in the glands of male mice. They are potent inducers of EGF. Thyroid hormones are also required for the maintenance of GCT cells and EGF levels, and are capable of inducing EGF expression in developing and adult animals. Using a labeled EGF nucleic acid probe, investigators at the Mount Sinai School of Medicine, New York, New York, have recently demonstrated that submandibular gland EGF mRNA concentrations increase during postnatal development of the gland and after the administration of testosterone or thyroid hormone.

Recently, it was reported that EGF mRNA is present in kidney as well as a number of other mouse tissues. A comparison of EGF gene regulation in submandibular gland and kidney revealed that kidney EGF mRNA levels also increase during the postnatal period. Opposite sex differences were observed, with submandibular gland levels being about 16-fold higher in the male than in the female and kidney levels being 2- to 4-fold higher in the female than in the male. Renal EGF mRNA concentrations were found to be less responsive to hormones than those in the submandibular gland.

In another study, these same investigators found that submandibular glands of senescent (26-28-month old) mice contained approximately 50% less EGF and 75% less EGF mRNA than those of mature (12-month old) males. Their findings indicate that these decreases in EGF and EGF mRNA seen in the entire gland with advanced age are caused by a widespread deterioration of the GCT cells themselves, which apparently can be reversed in many but not all GCT cells by stimulation with supraphysiologic doses of either triiodothyronine or dihydrotestosterone.

H05 Develop improved procedures for characterizing the molecular structure and function of the salivary proteins and other macromolecules important in oral health maintenance.

The enamel surfaces of erupted teeth are covered by a resilient, membranous film of salivary origin, the acquired pellicle. This covering, though only 10 µm thick, may protect the teeth against abrasion, erosion and demineralization; it also affects the attachment of cariogenic microorganisms to enamel. The pellicle is an acellular material, rich in proteins and glycoproteins selectively adsorbed from saliva; these include neutral and anionic proteins, and serous and mucous-type glycoproteins. Less is known about other salivary constituents which also exhibit affinity for the enamel-apatite surface such as lipids. The lipid content and composition of the enamel pellicle from caries-resistant (CR) and caries-susceptible (CS) subjects and their effect on its ability to retard the diffusion of lactic acid were recently investigated by grantees at the New York Medical College at Valhalla. Lipids accounted for 22.2 per cent of the dry weight of CR pellicle and 23.7 per cent of CS pellicle. The content of glycolipids in both groups was similar but CR pellicle contained 42 percent less neutral lipids and 31 per cent less phospholipids. CR lipids had a higher content of cholesterol, cholesterol esters and sphingomyelin, whereas CS pellicle was richer in free fatty acids and phosphatidylethanolamine. Retardation of lactic acid diffusion by CR pellicle was 45 per cent higher than by CS. Removal of lipids caused 50 per cent reduction in retardation by CR pellicle and 35 per cent by CS pellicle.

The retardation ability of pellicle appears to depend, at least in part, on its lipid constituents, because their removal resulted in increased lactic acid diffusion, and their re-association fully restored its retardation capacity. The difference in the retardation capacity between CR and CS pellicle cannot be attributed to the total lipid content, as this was similar for both. Probably the difference is due to compositional differences amongst the lipids, and the extent of their interaction with protein and glycoprotein constituents of pellicle. The permeability of other biological protective layers, such as that of gastric mucus to hydrophilic substances, depends upon the lipid composition. The hydrophobicity of the host tissue also plays a role in the initial attachment of bacteria to tooth surfaces. Thus, pellicle lipids may well not only control the extent of enamel demineralization by cariogenic acids, but also modulate the invasion of the tooth surfaces by bacteria.

Among the components of saliva that play a major role in the nonimmune protection of the oral cavity is mucus glycoprotein or mucin. This glycoprotein is known to interact with a variety of oral bacteria leading to their aggregation and clearance. This clearance mechanism, while successfully meeting the cariogenic bacterial challenge in the caries-resistant population, appears to be far less efficient in caries-susceptible people; yet no apparent differences in salivary mucins between these two groups have been demonstrated. In a recent study, investigators at the New York Medical College at Valhalla have provided evidence that the high-molecular-weight mucus glycoprotein from saliva of caries-resistant (CR) individuals differs from that of caries-susceptible (CS) subjects with respect to susceptibility to proteolytic degradation. While both glycoproteins in their intact forms were prone to proteolysis by pronase, the extent of their degradation was not equal. Extraction of associated lipids increased the extent of degradation in both cases, but the CS glycoprotein still remained less susceptible. After removal of associated lipids and covalently bound fatty acids, however, the susceptibility to pronase became essentially identical for both glycoproteins. Since the low-molecular-weight mucus glycoprotein is more efficient in bacterial aggregation, the CR individuals would have more of this glycoprotein available for bacterial clearance, while the CS individuals with their lower content might be unable to counteract the bacteria successfully. Although this conclusion is based on the results obtained with a proteinase which is not indigenous to saliva, similar differences may be expected in the oral cavity with respect to the susceptibility of mucus glycoprotein to proteolysis by salivary and bacterial proteinases.

Previous studies have identified both high- (MG1) and low- (MG2) molecular-weight mucins in human submandibular-sublingual saliva. Purification of the low-molecular-weight species has shown that mucin to be monomeric (200-250 kDa) and to comprise 31.4% protein, 67% carbohydrate (including 9.8% sialic acid), and 1.6% sulfate. The carbohydrate side chains of MG2 ranged in size from disaccharides to heptasaccharides. The sialic acid-containing trisaccharide of MG2 has been shown to interact with a lectin on the surface of Streptococcus sanguis, suggesting that one function of this mucin is to modulate the clearance of microorganisms from the oral cavity. In contrast, preliminary studies have suggested that MG1 might function in the formation of a protective semipermeable barrier (salivary pellicle) on enamel and/or mucosal surfaces. However, little information is available regarding the structure-function relationships of MG1. The present investigation by grantees at the State University of New York at Buffalo extends the analysis of human salivary mucins by comparing their biochemical and biophysical properties, and thus provides a basis for subsequent functional studies. MG1 was found to comprise 14.9% protein, 78.1% carbohydrate (including 4.0% sialic acid), and 7.0% sulfate. Carbohydrate units ranged in size from 4 to 16 residues. Collectively, the results of this study indicate that human submandibular-sublingual saliva contains two structurally distinct mucins. It is tempting to speculate further that the two salivary mucins may have distinct protective functions in the oral cavity. Several studies have demonstrated that MG2 can interact specifically with the oral bacteria Streptococcus mitis and S. sanguis and that such interactions are predicated, in part, by the sialic acid-containing oligosaccharides of this mucin. Conversely, MG1 possesses physicochemical properties that make it an attractive candidate for participation in enamel pellicle formation. Indeed, the low sialic acid/sulphate ratio of MG1 is consistent with previous findings that the sialic acid concentration of enamel pellicle is quite low. In this context, it has been recently shown that MG1 has a greater selectivity for hydroxyapatite than does MG2. It is, therefore, proposed that MG1 may serve a tissue-coating function, protecting the tooth surface as part of the initial acquired enamel pellicle. In contrast, sialic acid-enriched MG2 fails to adsorb in appreciable quantities to the tooth surface, making it available to interact with specific bacteria and help clear them from the oral cavity.

Human salivary secretions are supersaturated with respect to basic calcium phosphates but spontaneous precipitation of these salts from saliva, or surface-induced precipitation of calcium phosphates onto dental enamel, does not normally occur. This unexpected stability has been attributed to the inhibitory activities of two kinds of salivary phosphoproteins, statherin and the acidic, proline-rich phosphoproteins (PRP). Statherin inhibits primary (homogeneous nucleation followed by crystal growth) and secondary precipitation (nucleation occurs in the presence of crystals, followed by growth as, e.g., in seeded crystal growth) of calcium phosphates, while the PRP, although less effective as inhibitors of primary precipitation, are highly potent inhibitors of seeded crystal growth of calcium phosphate salts. These activities are of biological significance in that they act to provide a supersaturated, but stable, protective and reparative environment for the dental enamel. Several PRP have been identified and the amino-acid sequences of four of these proteins, including PRP-3, have been determined. The purpose of the present study by grantees at Forsyth Dental Center was to more completely define the specific groups or sequences responsible for inhibitory activity in seeded precipitation. To this end, the 30-residue, amino-terminal segment of PRP-3, which contains the two phosphoserines and 11 of the 13 carboxyl groups present in PRP-3, was obtained by tryptic digestion. This peptide, designated PRP-3(T1), was treated with thermolysin to give the monophosphopeptides, Val-PSer-Gln-Glu-Asp-Val-Pro and Leu-Val-Ile-Ser-Asp-Gly-Gly-Asp-PSer-Glu-Gln, and with alkaline phosphatase to give the dephosphorylated analog, PRP-3(T1) DP. The inhibitory activities of PRP-3(T1) and the derived peptides, a synthetic peptide, phosphoseryl-phosphoserine (PSer-PSer), and O-phosphoserine (PSer), were determined using an assay based on inhibition of hydroxyapatite seeded precipitation of calcium phosphate. Inhibitory activities, expressed as concentrations of inhibitors required to give standard inhibitory activities, were PRP-3(T1), 0.59 μM; PSer-PSer, 3.5 μM; the two monophosphopeptides, 29 and 32.5 μM; PRP-3(T1) DP, 56 μM; PSer, 329 μM. These results show the importance of the simultaneous presence of two phosphoserines in these precipitation inhibitors, and the contributions made by the carboxylic groups, but they also clearly show that a major part of the inhibitory activity is related to the structure of the intact molecule. Although inhibition of calcium phosphate precipitation in the assay system used in this study does not necessarily involve adsorption of PRP and the peptides onto hydroxyapatite, the previously shown marked relationship between the adsorption affinity constants and inhibition clearly suggests a close relationship between the two phenomena. This relationship will be investigated in further, more detailed studies, of the inhibitors examined here.

Individuals with diminished salivary gland function are subject to serious alterations of the oral mucosa, and may have difficulty with speech, mastication, and deglutition; in addition, a high rate of caries and attrition of the dentition may be observed. While the many causes of xerostomia and its proper diagnosis have been addressed in the recent literature, treatment is still limited to a narrow spectrum of substitute fluids to be taken ad libitum by the affected individual. While reports in the literature have assumed that a correlation exists between viscosity and oral lubrication, the latter parameter has not been adequately measured. The purpose of this report by grantees at the State University of New York at Buffalo was to measure human salivary lubrication by means of a device, previously described by Swann et al. and adapted by the grantees, that makes use of an oral hard tissue interface. Several commercially available salivary substitutes, including a mucin-based substitute, were assessed for their ability to lubricate in this system. Apparent viscosity determinations of these solutions were also performed and efforts made to find a correlation between these parameters. The results indicate that little correlation exists between these parameters and show that salivary lubrication of oral hard tissues may be a more complex phenomenon than previously believed. Saliva substitutes based on carboxymethylcellulose did not appear to lubricate biocompatible hard interfaces well and, therefore, might not protect against the rapid attrition observed in xerostomic individuals. In contrast, the mucin-based substitute proved to be a better lubricant with values comparable to whole human saliva. Studies are currently underway to define the biochemical and biophysical basis of salivary lubrication and viscosity between oral hard and soft tissue interfaces. It is hoped that this information may provide a rationale for the development of substitute fluids that may truly provide comfort and function for xerostomic patients.

H08 Develop reagents and background information necessary to understand and effectively use changes in saliva to diagnose and monitor specific disease processes, drug therapy, genetic defects, nutritional status and age-specific changes.

Sjögren's syndrome is a generalized, auto-immune, chronic inflammatory disease which affects the function of salivary and lacrimal glands in man. Its clinical manifestations involve a marked reduction in salivary and lacrimal flow because of progressive ductal changes that are due to lymphocytic infiltration and fibrosis of the glands. In the current classification, primary Sjögren's syndrome principally involves the salivary and lacrimal glands, whereas in secondary Sjögren's syndrome, xerostomia and xerophthalmia accompany rheumatoid arthritis or other connective-tissue diseases such as systemic lupus erythematosus. The dry mouth associated with this syndrome, apart from causing discomfort in eating and speaking, often leads to rampant caries and exacerbation of periodontal disease. Measurements of the changes in salivary flow rate and composition have been recognized for some time as valuable in differentiating Sjögren's syndrome from other diseases of the salivary glands. A marked rise in the concentration of sodium, chloride, immunoglobulins and lactoferrin and a decrease in phosphate have been reported in such saliva. Investigators at the New York Medical College at Valhalla have observed that salivary gland diseases also affect the content and composition of salivary lipids. Extraction of the dialysed and lyophilized parotid saliva of eight patients with Sjögren's syndrome yielded a level of lipid 2-times higher than in normal individuals. The saliva of such patients also had 3-times more glycolipid and 20-times more phospholipid than normal, but differences in the content of neutral lipids were less apparent. The neutral lipids, however, had higher proportions of glycerides, and lower proportions of cholesterol and cholesteryl esters than normal. Perhaps the most important finding of these studies is that parotid saliva from Sjögren's syndrome has a 20-times greater than normal content of phospholipids. If verified in a larger group of patients, the measurement of phospholipids in saliva might be used in the diagnosis of Sjögren's syndrome. This could be of particular value in differentiation of the syndrome from recurrent parotitis, in which the content of salivary phospholipids is similar to that of normal saliva.

α-Amylase, the most abundant enzyme in saliva, is formed primarily in the acinar cells, and less consistently in proximal cells of intercalated ducts. It is active in the initial stages of digestion. The predominant immunoglobulin in human mucosal secretions, including saliva, is secretory IgA (sIgA). Salivary sIgA can bind specifically to surface epitopes of oral flora, facilitating their clearance. As α-amylase is synthesized within the gland but sIgA is synthesized in plasma cells and reaches saliva by active transport, investigation of these two molecules permits assessment of several glandular compartments. Baseline information is essential for evaluation of salivary changes with age and disease. However, previous studies of age-changes in the composition of saliva have proved inconsistent. Investigators at the State University of New York at Buffalo have recently examined a large number of healthy, adult subjects from a well-defined population not using prescribed medication, whose parotid salivary flow rate, amount of protein and concentration of acidic proline-rich protein were previously found not to be affected by normal aging. These studies indicate that there are no age-related changes in α-amylase and sIgA concentration in gustatorily stimulated parotid saliva from a non-medicated, community-based population. Collectively, these recent findings and others those reported previously suggest that the aging process *per se* is not linked with physiological decline in human salivary glands.

ANNUAL REPORT - FY 1988

CRANIOFACIAL ANOMALIES, PAIN CONTROL AND BEHAVIORAL RESEARCH BRANCH

EXTRAMURAL PROGRAM, NIDR

More than 120 research projects received funding during 1988 to study the molecular and cellular etiology of craniofacial anomalies and to improve treatment of dentofacial malrelations and the more serious or extensive congenital defects such as cleft palate, hemifacial microsomia and craniosynostosis. Examples of studies include the hormonal control and the molecular and cellular events during palatal shelf reorientation and fusion to form the normal palate; mechanisms of bone remodelling; development and use of new techniques to interpret data on craniofacial growth and to assess the effects of orthodontic, orthognathic and other surgical treatments for anomalies. Approximately 100 projects were supported to study orofacial pain problems and behavioral factors involved in dental disorders. These include studies of the role of the trigeminal system in pain transmission; the pain associated with the temporomandibular joint and other types of myofascial pain and the impact of stress in its etiology and symptomatology; assessment of the safety of local dental anesthetics during pregnancy; neural mechanisms involved in the initiation and coordination of oral motor function.

C02 Uncover the mechanisms that govern interactions between regulatory genes and the extracellular environment.

C03 Characterize the chemical mediators that underlie cell-cell and cell-matrix interactions.

Formation of the mammalian secondary palate requires the forerunners of the palate, the wedge-shaped secondary palatal shelves, to undergo a reorientation from a vertical position on either side of the tongue to a horizontal position above it. There they meet in the midline, adhere and fuse to one another to form the normal secondary palate. Failure of this reorientation leads to cleft palate. Since the causes of abnormal formation of the palate are largely unknown, basic research into the biochemical regulatory mechanisms responsible for normal development of the palate is being supported to provide the framework for investigations into the etiology of palatal clefts. Investigators at the University of Michigan have shown that hyaluronate-mediated extracellular matrix expansion is crucial to shelf reorientation. However, expansion alone will not produce shelf remodeling, it must be directed. They have identified two specific regions of the shelf's epithelial perimeter, which show altered cell formation and increase in cell density during the course of reorientation. This increase is not attributable to cell division. They hypothesize that these local changes in epithelial cell packing direct the expansion of the palatal shelves such that correct remodelling is achieved. Using monoclonal antibodies to heparan sulfate proteoglycan they found that the distribution of the molecule around the surface of the epithelial cells is correlated with the shape of the cells. Cells which are cuboidal stain uniformly on all sides for the presence of the molecule; squamous, or flat, cells show no staining. Cells in the mid-oral region of the palatal shelf epithelium, a region shown to actively change cell shape and cell packing prior to shelf reorientation show interesting staining patterns. When cells are cuboidal they stain on all surfaces. As their packing density increases and their shape becomes columnar to pseudostratified columnar they lose the staining on their basal and lateral surfaces, except for small points of staining between the adjoining basal surfaces of two cells. The heparan sulfate proteoglycan interacts across the cell's membrane with the cytoskeleton; the cytoskeleton determines the cell's shape and in many instances its functional state. These results suggest that this molecule plays a role in determining whether or not epithelial cells change shape and packing in local regions of the palatal shelf epithelium during reorientation.

Recent findings suggest that normal growth and differentiation of embryonic palatal tissue depend on the presence and interaction of various hormones and growth factors regulating intracellular levels of cAMP. Studies from Thomas Jefferson University and other laboratories are directed toward identification of extracellular molecules triggering transmembrane signaling mechanisms utilized by embryonic palatal cells to elicit elevations of intracellular cAMP. During embryonic and fetal development, differentiating cells and tissues acquire a unique repertoire of hormone receptors. Catecholamine and prostaglandin receptors were demonstrated in the mouse embryonic palate during the period of tissue histodifferentiation. Their functionality was demonstrated by showing that embryonic palatal cells responded to both catecholamines and prostaglandin-E2 with dose-dependent accumulations of cAMP which modulated cell proliferation by delaying the initiation of DNA synthesis. Moreover, not only was embryonic palatal tissue shown to be capable of synthesizing several prostaglandins, but levels of the primary catecholamines (dopamine, norepinephrine, epinephrine) in embryonic palatal tissue altered quantitatively over the course of palatogenesis. Evidence was obtained that epidermal growth factor, or a related peptide, transforming growth factor-α (TGF-α), or fibroblast growth factor modulate development of the secondary palate.

These factors promote growth and differentiation of the epithelial and mesenchymal cells of the developing palate, in culture. Using immunohistochemical methods, TGF-α was localized in embryonic mesenchyme of neural crest origin that was destined to develop into a number of craniofacial structures including the secondary palate.

C10 Increase studies to identify and treat craniofacial anomalies before the consequences of abnormal growth and function occur.

Hemifacial microsomia is associated with unilateral hypoplasia of the facial and mandibular muscles and of the jaws, resulting in a skewed facial form. Subjects with this congenital defect are being studied at the University of California, San Francisco, to ascertain what neuromuscular compensatory mechanisms develop due to these anomalies and to improve orthopedic and surgical correction procedures. Normally, the masseter, temporalis and medial pterygoid muscles act in concert to close the jaws and the lateral pterygoid opens the jaws. These muscles are being assessed in subjects before and after treatment using computerized tomography, in which successive horizontal axial views have been taken and reconstructed in three-dimensional configurations. The individual muscles are assessed for area and volume on both affected and unaffected sides in order to provide an objective assessment of the potential change in muscle size during mandibular orthopedic treatment. The underdeveloped mandibular muscles increase in size with treatment, suggesting that the orthopedic approach stimulates the use of the underutilized muscle. In the most severely underdeveloped subjects, muscles such as the lateral pterygoid may be missing; some subjects may have only a remnant of the masseter muscle. The temporalis muscle always appears to develop in these subjects but may be underdeveloped and will respond to long-term treatment. Neuromuscular assessment of the mandibular and facial muscles has involved electromyographic recordings of the muscles during selected movements including mastication. The most severely affected hemifacial microsomia subjects with a missing or much reduced masseter muscle may use unusual recruitment patterns of the mandibular and facial muscles. These recruitment patterns associated with the congenital anomaly are also characterized by morphological data. Long-term mandibular orthopedic treatment with surgical repositioning of bony structures appears to alter this recruitment pattern for specific muscles as the mandible assumes a more midcenter and normal position from its previously laterally skewed resting posture. The long-term treatment is expected to alter this muscle pattern as it develops a new position for the mandible. In less severe hemifacial subjects with ankylosis of the temporomandibular joint, the muscle patterns are more normal despite the structural resistance, which prevents some normal movements of the mandible. These data suggest that such subjects have the neuromuscular capability of using the mandible without the ability to actually make the response.

Computerized tomography is also being used in the analysis and treatment planning for other craniofacial syndromes, such as craniosynostosis, where the area of pathology is greatest at the cranial base. Standard methods of analysis involving measurement and tracing of two dimensional cephalometric X-rays use the cranial base as a reference point, rendering them useless in these cases. Investigators at New York University's Institute of Reconstructive Plastic Surgery have devised three dimensional surgical simulation programs which operate on three-dimensional cephalograms and computerized tomographic models of the patient's facial anatomy. These programs allow the clinician to design the surgical procedure interactively. An optimization routine automatically positions the bone fragments to best fit normal position, corrected for age, race and sex based on matching landmark points. Future research in this area involves the development of more comprehensive facial analysis methods and the measurement of postoperative growth. Landmark point analysis is inadequate, particularly in the upper face, which is composed of smoothly curving surfaces with few distinct points. Analysis methods based on ridge curves and surfaces are being developed to improve surgical planning for the upper face and cranium. An electromagnetic sensor is used to measure rigid motions of dental models and eventually of intraoperative bone fragments.

Psycho-social considerations force the craniofacial team to correct severe facial anomalies long before the face is fully grown. It has been shown that personality development can be severely hindered if this approach is not taken. As a result, surgical planning becomes a four-dimensional problem. Three dimensional growth must be taken into account in arriving at a surgical plan. This requires that postoperative three dimensional growth be carefully measured and the results fed back into plans for subsequent corrective surgery on these patients. A facial laser scanner has been added to postoperative follow-up regimens to better track facial growth without exposure to ionizing radiation. It produces a complete facial scan from 120,000 points recorded in 5 seconds. The device allows collection of a three dimensional record of the facial surface at the time of each routine follow-up visit. This research continues to provide a more accurate and detailed understanding of the specific craniofacial anomalies and of methods for their successful treatment.

CS1 Develop active means of promoting research in craniofacial anomalies among established investigators, especially those in molecular biology, genetics, and epidemiology.

CS5 Promote and continue to support "centers of excellence" where there are interdisciplinary concentrations of basic scientists and clinicians.

Approximately five percent of babies born in the United States require treatment for various malformations. Single mutant genes and major chromosomal abnormalities are associated with about 13.5 percent of these malformations; combinations of genetic and environmental factors are implicated in a further 20 percent of cases. Three quarters of congenital malformations affect the head and neck and thus represent a major public health problem of interest to the NIDR. Cleft lip with or without cleft palate and isolated cleft palate are the most common and extensively studied birth defects of the craniofacial region, occurring in approximately one in every 600 live births. One in every 1,600 live babies in the United States are affected with craniofacial anomalies other than cleft lip and/or palate. Nearly fifty percent of the U.S. children are affected by dentofacial malrelations that warrant treatment and about five percent have sufficiently severe orthodontic or orthognathic problems that they are handicapped functionally and psychosocially. Genetic factors are also implicated in determining tooth size, morphology and number. At the molecular level, genes coding for proteins contributing to tooth structure and mineralization and also for production of collagens and other proteins of cartilage and bone have been characterized. Mutations of these genes may be responsible for inherited conditions such as amelogenesis- and osteogenesis-imperfecta.

Recent advances in molecular biology and genetics have provided the molecular basis for interpreting epidemiological and biochemical information on many human inherited disorders, permitting studies on their etiology, diagnosis, treatment and prevention. These advances have created opportunities for research on developmental disorders of genetic origin affecting human craniofacial structures.

In order to alert the research community to these opportunities and promote research capitalizing on these advances, three program initiatives were instituted. A state-of-the-art conference on the Molecular and Genetic Basis of Growth and Development was held at the NIH in February 1988. Topics included recent advances in recombinant DNA technology, the discovery of proto-oncogenes and their growth regulating products, advances in growth factors in general, recent progress towards understanding cell adhesion and cell substrate adhesion molecules, the discovery of homeotic regulatory genes, recent physico-chemical analyses of protein-DNA binding interactions, tissue-specific and steroid-specific gene regulatory elements, gap junctions and their role in intercellular communication, gene regulation, molecular determinants of morphogenesis, and molecular approaches to pattern and form determination. A more narrowly focussed international conference on Research Advances in Prenatal Craniofacial Development, jointly sponsored with the National Institute of Environmental Health Sciences was held at Research Triangle Park, North Carolina, in April 1988. Topics included cellular events including neural crest cell migration and craniofacial pattern formation, cell and tissue interactions during morphogenesis with special reference to bone and tooth formation, regulation of normal and abnormal craniofacial development, including the mechanism of action of teratogens with detailed discussion of retinoic acid embryopathy, genetic control of connective tissue protein production and the genetics of craniofacial growth patterns and susceptibility to clefting in animal and human populations. Finally, a Request for Applications was issued to solicit center grant applications, which will capitalize on recently developed techniques and information on molecular biology and genetics and create multidisciplinary teams to address problems of normal and abnormal human craniofacial development.

E04 Expand studies on bone formation and remodeling.

Bone is constantly turning over, although in health the amount of bone surface that is being remodeled is quite small. During much of adult life overall bone mass remains relatively constant due to the balance between formative and degradative processes. The uncoupling of these processes likely leads to the bone loss associated with periodontal disease, loss of the alveolar ridge in edentulous individuals, osteoporosis, orthodontic tooth movement and failures in fracture healing. NIDR supported investigators are examining the factors controlling these processes. Bone dissolution results from an orderly sequence of events; osteoclast precursors originating in the bone marrow, proliferate and differentiate, recognize the site for resorption, attach to the bone matrix and fuse to form multinucleated mature osteoclasts, which initiate bone resorption. Numerous factors such as calcitonin and parathyroid hormone, vitamin D and immune and extracellular matrix components appear to act in concert to control bone remodelling. Investigators at the Washington University are using monoclonal antibodies to recognize antigens, which are unique to the osteoclast cell surface. They can be used to localize and quantitate the osteoclasts as they are formed. The investigators have found that bone forming osteoblasts produce factors which promote osteoclast differentiation from bone marrow derived precursors. Noncollagenous bone matrix components promoted osteoclast precursor recruitment and their differentiation into osteoclasts. However, the matrix components they found capable of inducing osteoclast development were only active in the presence hydroxy-apatite, the mineral of bone.

In addition to the specific cell surface antigens, osteoclasts synthesize a number of characteristic hydrolytic enzymes, which are associated with their ability to resorb bone. Investigators at Yale University have shown that these enzymes are transported into a sealed off extracellular compartment adjacent to the bone, where resorption occurs in an environment which is actively acidified. The membrane which limits the acidified compartment is known as the ruffled border. It is rich in the enzyme ATPase, which produces protons creating the acidic environment in which calcium dissolves from the bone; other enzymes digest the organic bone matrix consisting of collagen and other bone proteins. These investigators are also studying how the acid is formed. They described two mechanisms to account for the movement of protons (acid) across the membrane. Using the energy from ATP the osteoclasts pump protons across the membrane, exchanging protons with potassium ions. The osteoclasts may also exchange sodium ions for potassium ions and thus set up a gradient to allow the efflux of calcium ions from the bone into the extracellular space.

E05 Expand studies of interceptive treatment aimed at growth modification.

E06 Study factors affecting the post-treatment stability of bones and teeth.

Orthodontic treatment, in addition to correcting the alignment of the teeth, is capable of producing changes in the shape of the face by influencing the growth of the bones of the head. Treatment can alter both the vertical height of the face and the flatness or convexity of the profile. Orthodontists classify patients according to facial form and attempt to design treatment to produce different kinds of changes in patients who start with different facial configurations. It is generally believed that different methods of treatment yield different kinds or magnitudes of change in facial form, but the relative effectiveness and long term effects of different treatment modalities have thus far received relatively little study.

In order to understand the effects of treatment strategies better, it is desirable to be able to distinguish between changes which result from the displacement of teeth within the jaws ("orthodontic" displacement) and those which result from displacement of the facial bones as a secondary consequence of forces applied to the teeth ("orthopedic" displacement). However, quantitative methods for distinguishing between these two types of treatment effect have not been available. In general, earlier studies have suffered from selection bias and inadequate measuring technologies. Investigators at the University of California at San Francisco have developed and refined specialized computer techniques and associated instruments for obtaining high quality physical data on growth and treatment effects. These methods consider the measurement of the head as a mapping problem and employ techniques borrowed from the field of satellite and aerial map making. These computer aided techniques for analyzing X-ray images of the face taken before, during and after treatment permit the clinician to differentiate orthodontic effects from orthopedic effects. A second approach is to use numerical data collected from X-ray images, photographs and plaster casts of the teeth, together with information collected from written treatment records to test statistically the appropriateness of various treatment strategies. The practical utility of this study is that it improves understanding of the modes of action of orthodontic appliances of different types and aids in choosing the therapeutic approach most likely to yield the most desirable facial form. These procedures have already resulted in the modification of some traditional ideas in the field of orthodontics. For instance, they have shown that the true variability in the response of different patients to the same kind of treatment is even greater than had formerly been believed. The investigators are developing a relatively large, well organized, and rapidly accessible relational data base containing information about a substantial and representative sample of treated and untreated orthodontic subjects. Such a data base will serve as a shared resource accessible to many investigators, who will be able to query it from different points of view as an aid in treatment planning.

G01 Conduct further studies of the trigeminal system with particular emphasis on pain transmission.

G09 Conduct basic and clinical studies of pain associated with the temporomandibular joint and other myofascial pain.

Investigators at the University of Toronto have demonstrated that electrical or chemical stimulation of the tongue, face, and tooth pulp all activate some of the same neurons in the trigeminal subnucleus caudalis. This is one of the major neuroanatomic sites where pain messages are relayed within the central nervous system. Single cell electrophysio-logical recordings showed that many of the pain-responsive (nociceptive) neurons in the caudalis which were responsive to stimulation of the oral mucosa also respond to stimulation of the canine tooth pulp and tongue muscles. This convergence of neuronal transmission into the caudalis helps explain why dental patients can have difficulty in accurately localizing orofacial and dental pain and experience referred pain. (Referred pain is perceived as occurring in a different body region than the region which is receiving noxious stimulation.)

These investigators are studying the central neural pathways and relay centers involved in processing of sensory information from the temporomandibular joint (TMJ). They demonstrated that electrical, mechanical, and chemical stimulation of the TMJ excites two types of neurons, classified as wide-dynamic range and nociceptive-specific, in the trigeminal nucleus caudalis. This is the first study in the literature documenting brain stem mechanisms involved in the relay of information from the TMJ.

They have also evaluated whether masticatory muscle hyperactivity can arise reflexly as a result of stimulation of TMJ afferents--a concept underlying much of the clinical literature on TMJ disorders and their treatment. They recorded electrical activity in the temporalis, genioglossus, and digastric muscles and found that electrical or chemical activation of TMJ afferents produced markedly increased activity of these masticatory muscles.

Studies such as these hold considerable potential for improving the differential diagnosis and treatment of the various conditions which lead to chronic pain in the jaw musculature and joint.

G08 Develop improved non-invasive methods of monitoring central nervous system, respiratory and cardiovascular effects of nitrous oxide and intravenous sedative drugs used in oral surgery and general dentistry.

Local dental anesthetics, such as lidocaine, have long been used during pregnancy with no apparent ill effects. However, several recent research reports noted possible behavioral deficits in human neonates and in rats exposed prenatally to lidocaine. Because lidocaine--and related drugs originally derived from cocaine--are known to affect the nervous system, cross the placental barrier, and because prenatal cocaine exposure is now known to cause developmental abnormalities, NIDR asked the FDA's National Center for Toxicological Research to evaluate the effects of prenatal lidocaine exposure in an animal (rat) model. The scientists studied how maternal exposure to various levels of lidocaine influenced indices of fetal wastage, birth defects, neurological functioning and behavior. Lidocaine was administered during mid and late pregnancy at several dosage levels ranging from minimal levels simulating dental exposure, up to near toxic levels.

These investigators found that even maternally toxic levels of lidocaine exposure produced no detectable morphological birth defects in the rat pups. The extensive behavioral tests administered also revealed no significant effects. These results indicate that prenatal exposure to lidocaine during dental treatment is not likely to pose risks to the developing conceptus.

G09 Conduct basic and clinical studies of pain associated with the temporomandibular joint and other myofascial pain.

An NIDR-supported longitudinal study at the University of Minnesota is studying signs and symptoms of temporomandibular dysfunction (TMD) among undergraduate nursing students. Using quite inclusive criteria for TMD, the investigators reported that only 29 percent of their sample was classified as "normal" (i.e., showing no signs of TMD; 33 percent showed signs of myofascial pain dysfunction and 37 percent showed signs of some level of internal derangement of the TMJ.

Further, of those initially classified as showing TMD, approximately 20 percent were classified as normal at an examination 18 months later--a finding which may indicate either unreliability in the assessment procedures employed (a somewhat more rigorous, systematic use of measures commonly used in clinical dental practice with TMD patients) or actual changes in TM function over time.

Comparable patterns of fluctuation over time in TMD signs and symptoms were recently found in a longitudinal study of adults insured through a health maintenance organization (HMO) in Washington State. University of Washington dental scientists designed a survey on which a large sample of HMO enrollees reported all signs or symptoms of TMD. Thorough clinical examinations were conducted on all subjects reporting TMD signs or symptoms, and these examinations were repeated after one year.

Examination findings provided the basis for classification according to the widely-utilized diagnostic criteria proposed by Eversole and Machado (JAMA, 1985). Classification categories include: myofascial pain dysfunction (MPD); internal derangement of the TMJ; degenerative joint disease; and normal.

Of subjects initially classified as showing MPD, 26 percent were classified as normal at one-year follow-up. Similarly 25 percent of those initially classified as showing internal derangement of the TMJ were classified as "normal" at follow-up examination, despite the fact that very few had received any treatment.

In general, the results provided no evidence that--at least over the one year interval studied--progression of signs and symptoms could be expected in any substantial proportion of patients. These patients will be examined periodically over the next five years to determine whether specific risk factors can be identified which predict progression in some patients of this puzzling, still controversial, disorder.

G10 Study the role of anxiety, stress, and other psychosocial factors, in relation to the etiology and symptomatology of a variety of motor dysfunctions and pain disorders.

M02 Identify how fluctuations in emotional states are associated with oral psychophysiological responses and with oral pathologies.

Investigators at the University of Pittsburgh have evaluated two alternative etiologic models of temporomandibular disorder (TMD): namely, the view that the disorder results primarily from a structural abnormality vs the view that it is primarily a psychophysiological stress-related disorder resulting from muscle hyperactivity. All patients studied fit well-defined inclusionary and exclusionary criteria and received an extensive series of clinical, diagnostic, and behavioral assessments. The findings of this study are summarized below:

- computerized tomography (CT) scan-identified TMJ abnormalities were unrelated to baseline masticatory EMG (electromyelogram) levels, a measure of activity in the muscles;
- patients with structural TMJ abnormalities as identified by CT did not show different levels of muscle activity as detected by EMG under either resting or mildly stressful conditions when compared with healthy controls.
- patients with TMD pain did not show significantly higher resting baseline EMG levels than did healthy controls, and
- compared to normal controls, TMD pain patients demonstrated greater response to mild experimental stress (such as doing mental arithmetic) in their masticatory muscles than did normal controls.

These results support the view that TMD pain patients respond to stressful environmental stimuli with site-specific muscular hyperactivity and suggest that for many--though certainly not all--of these pain patients psychophysiological, rather than structural, factors may be primary.

This same team of investigators has developed and cross-validated an empirically derived taxonomy of chronic pain patients which integrates medical, psychosocial, and behavioral measures. Using factor analytic techniques they have identified symptom clusters that occur simultaneously in pain clinic patients. They have demonstrated that most pain clinic patients can be classified reliably into several profile types and have found that TMD and low back pain patients can be classified with comparable reliability using this empirically derived schema for classification.

G13 Elucidate the role of cortical and subcortical structures in guiding and monitoring complex facial movements.

Ongoing research at the University of California-Los Angeles is determining how components of the central nervous system (CNS) responsible for sensing internal state conditions related to the need for water or food intake activate motor control brain stem networks that control drinking and feeding behaviors.

As a category for study, neural mechanisms involved in the initiation and coordination of oral motor behaviors--despite the critical importance of such behaviors for survival--have received limited scientific attention as compared with studies of the neural control of eye movements or limb movements.

Oral motor function plays an important role clinically in patients' adaptations to oral prostheses, such as implants or removable dentures; it can potentially be compromised or improved by dental interventions such as orthodontic treatment or orthagnathic surgery; and it has--as yet poorly understood--relationships to various orofacial pain conditions.

Experiments by these UCLA dental scientists have demonstrated for the first time that two distinct neural networks govern rhythmic jaw movements (chewing) and some of the movements involved in drinking (lapping-like altered rhythmic jaw movements). Having earlier found that injection of the drug apomorphine produces these lapping-like movements in the anesthetized guinea pig, these investigators have now demonstrated that administration of a neuroleptic drug (Haloperidol) used clinically in the management of psychotic disorders selectively blocks the occurrence of lapping, but not chewing, jaw movements. This is of interest because haloperidol is known to block a particular type of dopamine (D_2) receptor. This and other studies by this group suggest that lapping behaviors depend on activation of dopaminergic receptors.

In contrast, intravenous injection of the regulatory peptide, angiotensin II, evokes both lapping and chewing movements. Since earlier studies have shown that injection of this drug induces drinking even in water-sated animals, the investigators hypothesize that this regulatory peptide activates a dopaminergic circuit responsible for the activation of brain stem neural networks that produce coordination of the trigeminal and hypoglossal motoneuron pools necessary to accomplish the motor activities involved in drinking liquids.

These basic studies on the neural basis of biologically significant oral motor behaviors have already increased understanding of how the CNS controls complex motor activities. They are, additionally, establishing a foundation for increasing understanding of neurochemical and neurophysiological connections between motivational states, such as thirst and hunger, and related complex oral motor behaviors.

ANNUAL REPORT - FY 1988

PERIODONTAL & SOFT TISSUE DISEASES RESEARCH BRANCH

EXTRAMURAL PROGRAM, NIDR

During FY 1988, the Periodontal and Soft Tissue Diseases Branch made 120 awards for research on periodontal diseases and 45 awards for research on soft tissue diseases. The cost of the periodontal research plus related projects supported in the Research Centers on Oral Biology totalled approximately $23 million. Research on soft tissue diseases consumed approximately $7.0 million. Training and development support related to periodontal and soft tissue diseases was provided by NIDR Extramural Programs for approximately 100 individuals at a cost of $5.0 million. Thus, total expenditures for these activities approached $35.0 million.

Selected research highlights in the periodontal area consisted of laboratory, animal and human studies with clear emphasis on clinical orientation. Included was a project implicating a Streptococcus species in refractory periodontitis, two genetic projects indicating the importance of genetic factors in susceptibility to periodontitis, studies on the mechanisms of collagen fiber degradation in disease, tests of a new method of detecting collagen destruction, assessment of the feasibility of diagnosis based on microbial identification, and a promising preliminary report on treatment by means of a new, biodegradable, antibacterial slow-release system. Research on soft tissue diseases brought out findings on the epidemiology of oral lesions in several HIV-infected cohorts, including data on the high prevalence of candidiasis and hairy leukoplakia in infected adults, and the early appearance of gingivitis, and periodontitis in some HIV-infected individuals. Also included were oral cancer studies on the basic cellular biology of stem cells which can become malignant, and studies on the role of human papilloma virus and herpes simples virus in the etiology of oral cancer. Brief descriptions of these projects are presented below under the classifications developed for the NIDR Long Range Plan.

B01 Identify the microbial species that cause various forms of periodontal diseases.

Research over the past two decades from laboratories in different parts of the world has strongly implicated Gram-negative, anaerobic oral bacteria as the culprits in almost every type of periodontal disease. However, recent studies at the University of Florida indicate that at least one Gram-positive organism, Streptococcus intermedius may be causative in some patients who are refractory to standard periodontal therapy. Refractory patients are defined as those who sustain continual episodes of periodontal breakdown, in spite of surgery, antibiotics and repeated scaling. In twelve such patients, active and control periodontal sites both showed a mostly Gram-positive flora during quiescent periods, which did not differ significantly between the two types. However, when breakdown began to occur, the active sites became populated by Gram-positive organisms not present in the inactive sites, and the predominant organism was S. intermedius. These patients were treated with Augmentin and have subsequently been monitored clinically and microbiologically for 24 months so far. During this period, except for an occasional positive culture, S. intermedius has remained essentially eliminated and the clinical condition of the formerly active sites has been indistinguishable from that of the control periodontal sites. These findings are consistent with results obtained by investigators at Forsyth Dental Center in an earlier study which showed S. intermedius as a predominant organism in 10 of 13 refractory patients.

BO2 Investigate genetic, biochemical and stress factors influencing susceptibility to the periodontal diseases.

The Role of Genetics in Periodontal Disease: Although clinicians have long been prone to make anecdotal observations linking periodontal disease susceptibility to hereditary factors, there has been little definitive information on this subject and no systematic studies have been carried out to assess the role of genetics in periodontal diseases. In the past two decades, however, considerable effort has been devoted to a familial and apparently genetic condition known as juvenile periodontitis, and this research has encouraged investigators to attack the problem of genetic susceptibility to periodontal diseases in the general population. Early findings from two such studies are presented in the paragraphs to follow.

The study of twins provides an appropriate tool which enables investigators to make an initial estimate of the importance of genetic factors in a human disease or condition. Findings from such studies can now be analyzed by means of several new statistical study models.

In a study at the University of Minnesota, periodontal disease findings are being compared in identical and fraternal twins, and the degree of periodontal similarity measured and its significance ascertained. The working assumption of the investigators is that the degree of similarity between monozygous twins is twice that between dizygous twins. So far the investigators have analyzed data from 30 pairs of Caucasian twins, which included 14 monozygous pairs (6 pairs reared apart and 8 reared together), and 16 dizygous pairs (3 pairs reared apart and 13 together). The average age was 42 years. . Analysis of data on attachment level, pocket probing depth, gingival and bleeding indices indicates that genetic factors may be a strong component in the etiology of chronic destructive adult periodontitis. The heritability estimates for the characteristics measured were all calculated to be greater than 50%. On the other hand, there was no indication that plaque index, calculus index or plaque microbial composition were influenced by genetic factors. Continued study of genetic influences may enable clinicians to identify individuals with genetic susceptibility early in life and initiate programs of prevention.

Immunogenetic factors appear to be responsible for a recently discovered difference in susceptibility to juvenile periodontitis between a black and a white population of juvenile periodontitis families being studied at Virginia Commonwealth University. The purpose of the study was to determine the effect of racial factors on antibody reactivity to the organism *Actinobacillus actinomycetemcomitans* strain Y4 (*AAY4*) for subjects with different clinical manifestations. A radioimmunoassay was used to measure antibody activity. In family members without periodontitis, members with localized juvenile periodontitis, and members with severe generalized periodontitis, higher antibody titers to AAY4 were found for black subjects than for whites. It was also noted clinically, that black subjects with juvenile periodontitis showed a lower incidence of progression to the generalized form of the disease than their white counterparts. Thus, the data suggest that the stronger immune response to the allegedly causative organism AAY4 observed in the black group may protect them against further progression of localized juvenile periodontitis to the generalized form of the disease. The authors speculate that there may be differences in the immune response genes between blacks and whites.

BO4 Elucidate the mechanisms leading to the destruction of soft tissue and bone in the periodontal diseases.

To determine how periodontal tissue destruction takes place in disease, investigators have undertaken extensive studies on both the bacterial plaque and the underlying periodontium. Numerous studies have shown that the bacterial cells and the tissue cells possess a variety of mechanisms which could be involved in the events leading to the breakdown of the collagen fibers and bone which support the teeth. According to recent findings at Forsyth Dental Center and University of Alabama, one possible mechanism is that specific bacteria bind with gingival crevice epithelial cells, the cells lining periodontal pockets, and stimulate these normally protective host cells to secrete collagenase enzyme which initiates dissolution of the structural collagen fibers which hold the teeth firm.

Having developed a special method of quantitatively assessing the attachment of oral bacteria to oral mucosa, investigators at Forsyth took crevicular epithelial scrapings and samples of unattached microbial plaque from diseased periodontal sites in 8 patients. They then determined the predominant cultivable organisms attached to the gingival crevice epithelial cells and the predominant flora in unattached plaque. The results showed that the mean percentages of the specific organisms *Bacteroides gingivalis*, *B. intermedius*, and *Peptostreptococcus micros* attached to the epithelial cells was 5- to 20-fold higher than the percentages of these organisms in the unattached plaque. Apparently the organisms are selectively binding to the epithelial cells and presumably interacting with them. In earlier studies at the University of Alabama, it had been shown that *B. gingivalis* elaborates a substance which stimulates epithelial cells to secrete collagenase. Thus, it would appear that *B. gingivalis*, by interacting intimately with epithelial and stimulating them to produce a degradative enzyme, can cause periodontal destruction without being invasive.

In related studies, the Alabama investigators have isolated the collagenase enzyme from the epithelial cells that initiates the degradation of the connective tissues, and are currently studying its characteristics and how it is activated and regulated. They are also examining by means of recombinant techniques a collagenase enzyme from fibroblasts that is very similar to the enzyme secreted by the epithelial cells. Continuation of these basic molecular biology studies may lead to an understanding of how to prevent and control the connective tissue destruction which occurs in a number of chronic diseases.

B06 Develop safe, sensitive, objective tests of disease activity and rates of progression.

Gingival Crevice Fluid Collagen Assay: Since collagen fiber destruction is an essential feature of periodontal disease, investigators have sought to develop methods to detect and measure collagen or its products in the gingival pocket, or crevice fluid (GCF) as a means of determining the presence of local disease, especially in its early stages. An extremely promising method for achieving this aim is a new immunoassay that investigators at SUNY Stony Brook have developed and tested in beagle dogs with periodontal disease. They have compared the new immuno-nitrocellulose strip (INS) method to the standard inhibition ELISA method, and showed that the INS method is distinctly superior. The investigators believe that the new method could become a popular chairside technique, which would provide results within one hour without requiring expensive, complicated equipment. The preliminary results are outlined below.

Measurements were made on GCF samples collected twice during a 2-week period from periodontal sites in beagle dogs, and assayed for collagen or its breakdown products by the inhibition ELISA method and the INS method. Thirteen sites were healthy, ten exhibited gingivitis, and eighteen, periodontitis. The amounts detected by both methods were correlated with increasing severity of gingivitis and increasing pocket depth. However, the sensitivity of the INS method was much greater than the ELISA method. The INS method detected as little as 50 picograms in a single GCF sample, whereas the lower limit for the inhibition ELISA method was 1 nanogram. Moreover, two pockets classified as healthy clinically were negative for collagen when tested by the ELISA method, but showed the presence of a small amount of collagen when the INS method was used. Thus, the new technique seems highly promising as a rapid, convenient and sensitive assay for early periodontal disease activity that could find widespread applicability in the management of dental patients.

Diagnosis By Specific Organisms: As increasing evidence has accumulated implicating specific microorganisms in periodontal disease, there has been a growing belief on the part of many investigators that periodontal disease could be diagnosed and perhaps predicted by determining whether certain putative pathogens were present. In recent analytical studies at the University of Pennsylvania using the Bayesian decision theory approach, data on the occurrence of three such organisms, *Bacteroides gingivalis*, *B. intermedius*, and *Actinobacillus actinomycetemcomitans*(*Aa*) have been used to find out whether the presence of these organisms meant periodontal disease was present or could be predicted. The conclusion reached by the authors was that a single and accurate test to demonstrate etiologic agents and predict outcome disease in the general population may not be feasible for periodontal disease, because there is a relatively low incidence of active periodontitis and the microbial detection approach so far does not have high disease specificity.

However, the studies also indicated that microbiological diagnosis may be very helpful in predicting destructive periodontal disease in high-risk groups. Such groups may include young children in families with localized juvenile periodontitis, diabetics, adults with refractory periodontitis, AIDS patients, and individuals with neutrophil defects. For example, *Aa* seems to be predictive for juvenile periodontitis, and a particularly invasive strain of *B. gingivalis* has been linked to adult diabetics.

BO8 Develop more effective means to control or eliminate periodontal disease-causing bacteria.

A long sought goal for the treatment and prevention of periodontal disease is a suitable drug delivery system for antibacterial agents. A concept which remains promising is the principle of controlled release, whereby small amounts of the agent can act locally for an extended period. However, previously developed systems for periodontal pockets have had three disadvantages: too much time is required to place the antibacterial material, the drug release period is relatively short, and the carrier material is not biodegradable.

Recently, investigators at Membrane Technology and the University of California at San Francisco have developed a new delivery system which consists of polylactic-glycolic acid microcapsules containing tetracycline hydrochloride suspended in a hydrogel matrix. The new preparation can be easily and quickly applied with a syringe into the periodontal pocket, where it releases tetracycline for a 4 to 10 day period. Moreover, all of the components of the system are biodegradable. In preliminary tests in monkeys with gingivitis, effective tetracycline concentrations of 30 ppm were maintained and the gingivitis resolved within 4 days. Microorganisms are completely inhibited at 30 ppm.

In the next phase of these studies, the investigators will use the known periodontal pathogen Bacteroides gingivalis to induce ligature periodontitis in specific periodontal pockets in monkeys, and subsequently will treat the infection with the controlled release formulation. This approach will enable them to closely correlate the clinical changes with the concentrations of tetracycline and numbers of microorganisms in the pocket. The investigators believe that the new treatment will be especially useful in managing the difficult periodontal problem of furcation involvement (furcations are the fork-like areas where the roots divide).

F02 Study the role of stem cells and the mechanisms that regulate their proliferation and subsequent mucosal cell differentiation.

A subpopulation of slowly-cycling epithelial cells believed to be stem cells may serve as target cells for neoplastic transformation. It has been proposed by investigators at the University of Iowa that these slowly-cycling cells produce cells which are committed to differentiation, but before they differentiate, they undergo a series of amplification divisions. Increased divisions by the committed amplifying cells, or by the original stem cells themselves could result in epithelial hyperplasia.

To examine the role of the stem cells in chemically induced hyperplasia, the Iowa investigators labeled groups of C3H mice with 3HTdR or bromodeoxyuridine (BrdU) and 30 days later, their ears were treated with various concentrations of the hyperplasia-promoting agent TPA, or other hyperplasiogenic or non-hyperplasiogenic control substances. Tissues treated with TPA became hyperplastic, exhibiting a 2-6 fold increase in cell proliferation, which appeared to be a reflection of increased stem cell division rather than increased amplification division. Other inducing agents produced hyperplasia by increasing the amplification divisions. These studies are expected to lead to a better understanding of the mechanisms involved in carcinogenesis.

F06 Examine the roles of microorganisms such as viruses, fungi and other environmental host factors in the development of neoplastic lesions of the oral soft tissues.

Potential etiologic factors in oral cancer include Human Papillomaviruses (HPV), Herpes Simplex Viruses (HSV), tobacco and its byproducts. Papillomaviruses as a family have been known to cause a variety of abnormalities in the growth and differentiation of epithelial cells in skin, oral, and genital tissues. Although some HPV types have been associated with benign changes, others have been linked with malignancy, particularly in cervical carcinomas, which show evidence of HPV in 85% of the cases. HPV infection is known to occur in oral tissues, but its role in the development of abnormal growths of oral tissues has not been well investigated.

One approach currently being followed at the University of North Carolina is to look for signs of HPV in tissue sections of epithelial abnormalities. In these studies, scientists are searching for virus genomes of specific types and for other specific products which would indicate the presence of HPV. This approach is based on previous studies which consistently showed specific virus products in cervical cancers and established that these products could play a role in the conversion of normal cell cultures to a cancer-like state.

In another project, investigators at the University of Texas in Houston have studied 25 fresh specimens of oral cancer tissue from patients and have detected Papillomaviruses in 50% of them. Subsequently, the patients were entered into a long-term follow-up study, to determine if the presence of the virus indicates a favorable or unfavorable prognosis. Papillomavirus-infected cell cultures are also being studied to determine specifically whether the virus induces malignant changes in the cells in vitro and how the virus affects the behavior of the cells in general. Such studies may lead to the development of agent to inhibit the deleterious effects of HPV.

Patients with oral cancer consistently demonstrate higher levels of antibody to HSV than patients without oral cancer, indicating exposure, but the role of the virus is still not known. The aim of another investigation at the University of Texas in Houston is to understand how HSV could cause cells to become malignant. The working hypotheses is that HSV causes tumors in the same way that chemical carcinogens do, by inducing permanent mutations in cells. To determine the types of mutations that are made, investigators have placed small fragments of DNA (shuttle vectors) into monkey kidney cells, added HSV to the cells, then recovered the fragments and determined the change in their DNA sequence. The findings have shown that HSV makes three kinds of mutations: small base substitutions, small deletions of DNA, and large insertions of DNA excised from the chromosomes of the cells. The significance of these large insertions is that they may represent a target in the cell chromosomes for the virus. Future studies will attempt to identify the DNA sequence of the insertions and determine its role.

F99 Examine the role of smokeless tobacco on soft and hard tissues of the oral cavity, systemic effects and the role of viruses as cofactors in the etiology of smokeless tobacco lesions.

Current studies are focussing on the epidemiology of smokeless tobacco lesions in groups at high risk. A major undertaking recently initiated at the University of California is a project on the oral and other health effect of smokeleless tobacco in baseball players. This study will describe the patterns and prevalence of tobacco use and associated oral lesions in seven major league baseball teams and their associated minor league teams. The study is analyzing the relationship of smokeless tobacco to oral mucosal lesions, including ulcerations, erythema, leukoplakia, gingivitis, gingival recession, periodontitis, dental caries, enamel erosion, tooth staining and salivary gland abnormalities. The role of viral cofactors in the development of smokeless tobacco lesions and the systemic effects are also under study. So far the investigators have conducted initial spring training examinations and obtained baseline data on 1100 baseball players, and the data are being analyzed. The findings from this study are expected to be of great help in designing educational and behavioral programs for prevention of smokeless tobacco use, especially young athletes.

F99 Research on the oral manifestations of AIDS syndrome is currently focussed on epidemiology and pathogenesis of the oral lesions which occur and their clinical management. It is now recognized that oral lesions are serious and important complications of the immune suppression characteristic of this syndrome.

Epidemiology/Oral Manifestations of AIDS Oral candidiasis is the most frequently encountered opportunistic lesion found in HIV positive individuals. It is caused by the fungus Candida albicans. It is already known that parotid and submandibular saliva contain an abundance of histidine-rich proteins (HRPs), which are potent antifungal agents. These proteins serve as nonimmune natural host defense factors which regulate the numbers of C. albicans in the oral cavity. Investigators at SUNY Stony Brook are currently conducting detailed studies on the structure of these proteins in an effort to understand their function and their potential role, if any, in modifying the course of AIDS. The investigators speculate that HIV-seropositive patients who develop oral candidiasis early may have lower salivary levels of HRP, those who develop candidiasis later.

Epidemiology/Oral Manifestations of AIDS In a cross sectional epidemiologic study at the University of Washington in Seattle, 567 homosexual males were screened for oral lesions prior to determination of HIV status. Twenty-one percent were HIV-positive and 79% HIV-negative. At least one oral lesion was found in 29% of the HIV-positive subjects as compared to 8.5% of the HIV-negative subjects. By far, the two most prevalent lesions found were candidiasis and hairy leukoplakia. Two cases of Kaposi's sarcoma were also diagnosed, one in a young man who had no other signs or symptoms of HIV infection or prior knowledge that he might be infected with the virus. Analysis of the data showed that subjects with one or more oral lesions at screening were 4.4 times as likely to be identified as HIV-seropositive than subjects with no oral lesions. These findings strongly indicate that oral screening examinations should be carried out in subjects at high risk for HIV-associated complications, so that diagnostic and preventive measures can be instituted as early as possible.

In a multifaceted project on the epidemiology and pathogenesis of the oral manifestations of AIDS, investigators at the University of California in San Francisco are examining several cohorts of individuals to determine the extent and variety of oral lesions. In one initial survey of an adult cohort, the most prevalent oral lesion was hairy leukoplakia, which was observed in 15% of the patients examined. Candidiasis, at 10%, was the second most frequently occurring lesion. Other lesions included aphthous ulcers and oral Kaposi's sarcoma. Both low T4 cells and increased duration of infection were associated with the presence of the oral lesions.

In a cohort of children, the nature and prevalence of oral lesions were studied in seropositive children born to HIV-infected mothers. Sixteen children had oral lesions, whereas 8 children had normal oral examinations. The oral lesions included candidiasis, the most common lesion observed, and the first confirmed case of hairy leukoplakia in a child.

A third component of this project was focussed on the clinical features and tissue pathology of the salivary glands in HIV-infected patients. Five patients exhibited parotid gland enlargement and complained of dry mouth and dry eyes. Subsequent biopsies of the labial salivary glands showed focal chronic inflammation with features of Sjögren's syndrome.

Periodontal Disease in AIDS Patients During the past year, investigators at the University of California in San Francisco have identified two periodontal conditions associated with AIDS: an atypical gingivitis (HIV-G); and a rapidly progressive, severe periodontitis (HIV-P). Findings from the large epidemiologic survey they conducted suggest that these periodontal lesions may affect as many as 40-50% of the HIV-seropositive population. Moreover, HIV-G may be one of the earliest manifestations of HIV-infection, since this condition has frequently been observed before a drop in T4 count occurred and before any other oral lesions developed. Recognition of HIV-P was based on clinical observations in 83 patients who had severe periodontitis, but no other signs of symptoms of HIV infection. Upon questioning, these patients admitted to being homosexual and ultimately tested HIV-seropositive.

HIV-G is characterized by redness and edema of the free gingival margin accompanied by petechiae-like and/or diffuse red lesions of the attached gingiva, oral mucosa or both. HIV-G is usually resistant to conventional therapy. HIV-P resembles acute necrotizing ulcerative gingivitis (ANUG) superimposed upon a rapidly progressive severe periodontitis. However, the rapid and severe destruction of supporting bone in HIV-P clearly distinguishes this lesion from ANUG. The major clinical features include necrosis, ulceration and cratering of gingival tissue, frequently extending into the oral mucosa, extremely rapid bone loss, severe "deep" pain, and spontaneous and/or nocturnal gingival bleeding. In some instance there is denudation of bone and sequestration.

Microbiology studies carried out so far in a group of 64 patients, which included 45 seropositive individuals indicate that the organisms associated with periodontal disease in HIV-infected patients include the Gram-negative anaerobes Bacteroides gingivalis, B. intermedius, Fusobacterium nucleatum, and A. actinomycetemcomitans. These organisms were found in comparable frequencies and concentrations in both HIV-G and HIV-P lesions. Thus, the microbiology of the HIV lesions is similar to the Gram-negative anaerobic, flora found in HIV-seronegative patients. One exception, however, is Candida albicans, which was found in 74% of HIV-P sites and 49% of HIV-G sites. This fungal organism is not typically associated with subgingival plaque and periodontal disease. Anecdotal observations suggest that complete resolution of HIV-associated periodontal lesions is difficult unless successful antifungal therapy is included in the overall treatment provided. Subsequent studies will attempt to confirm these findings and correlate them with the clinical and immune status of the patients.

INTRAMURAL RESEARCH PROGRAM

ANNUAL REPORT OF THE BONE RESEARCH BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Bone Research Branch encompasses programs in cell biology, molecular biology, protein biochemistry and molecular biophysics. Its central focus is on the structure, metabolism and pathology of bone, cartilage and related connective tissue. It is the only NIH Branch or Laboratory devoted to bone research. A number of significant research advances were achieved this year. These are detailed below.

Skeletal Biology Section

The features that distinguish one connective tissue from the other arise from the cells and extracellular matrices that comprise them. The major functions of the bone-forming cells are, in fact, to synthesize, secrete, organize and mineralize a particular set of primary gene products, the precise proportions of which are unique to the tissue. All of the structural, biomechanical and metabolic properties of bone either originate directly or are modulated by these gene products. Further, over twenty different hormones, vitamins and growth factors are known to regulate bone metabolism. In normal circumstances, bone formation is tightly regulated by the concerted efforts of these regulatory agents. Imbalances in either the intrinsic features of bone turnover or the regulatory systems that control it lead to heritable and acquired diseases of the skeleton. These diseases are marked by changes in bone cell synthetic patterns that can be followed experimentally. Studies of such phenomena are organized in the Skeletal Biology Section (SBS), Dr. John D. Termine, Chief, along three interdependent disciplinary lines of investigation; protein chemistry, cellular biochemistry and molecular biology.

The protein chemistry group, headed by Dr. Larry W. Fisher, continued to have good success in the production of monospecific antisera to synthetic peptides of NH_2 and COOH- terminal sequences of bone proteins. These include: a variety of bone osteonectins from different animal species; human bone sialoproteins I (osteopontin) and II; human bone proteoglycans I and II; alkaline phosphatase; and osteocalcin (bone gla protein). Antisera to the N- and C- terminal sequences of human procollagen $\alpha1(I)$ were used to develop radioimmunoassays (in collaboration with the Arthritis Unit, Massachusetts General Hospital, Harvard Medical School) to study the turnover of type I collagen in blood and urine from patients suffering from metabolic bone disease. These antisera, along with those against human osteonectin were used to follow the course of advancing periodontal disease in a collaboration with the Department of Periodontology, University of Maryland Dental School. Preliminary results suggest that proteoglycan and osteonectin proteins (or their fragments) are present in human crevicular fluid at increasing levels with the progression of periodontitis in both young and adult human patients.

In a combined effort by Dr. Fisher and the Section's molecular biology group, headed by Dr. Marian F. Young, separate cDNA encoding both bone proteoglycan I and II were cloned from a human bone cell expression library. The cDNA encoding human bone proteoglycan II extended data published earlier for human placental proteoglycan II (decorin) at the 3' end by approximately 100 bp (base pairs). The cDNA encoding human bone proteoglycan I (biglycanin) was unique and showed that this connective tissue proteoglycan was a distinct but related gene product to proteoglycan II. The derived amino acid sequence of proteoglycan I shows remarkable homology to a wide variety of non-connective tissue related proteins, as well. These include two drosophila morphometric regulator proteins, toll and chaoptin, yeast adenylate cyclase, platelet glycoprotein Ib (a receptor for von Willebrand factor), and serum leucine-rich glycoprotein. The homology noted for all these proteins results in a 24 amino acid repeat that is postulated to allow these homologous molecules to interact with other proteins and/or cell membranes. These findings extend the possible biological roles for the core proteins of the two small major proteoglycans of most connective tissue and suggest multiple potential interactions for such molecules in their parent tissue systems.

In separate experiments, the Molecular Biology Group characterized the properties of bone osteonectin. Osteonectin contains several structural domains some of which appear to be involved in Ca^{2+} and hydroxyapatite binding. Using recombinant cDNA as probe, the regulation of osteonectin expression was studied. A comparison of osteonectin protein and mRNA levels in the developing limb bud indicates the protein is regulated by post-transcriptional mechanisms in proliferating cartilage. In isolated cells, on the other hand, osteonectin mRNA transcription is thought to be affected by TGF-ß, PTH, and 1,25-dihydroxyvitamin D_3. To understand the basis of this regulation at the nuclear level, genomic DNA encoding the promoter was isolated. DNA sequence analysis revealed the presence of multiple DNA motifs known to be involved in the activation of many genes. The promoter also possesses an unusual S1 endonuclease sensitive site which, when isolated, promoted transcription of a marker gene in transfected bone cells. These results indicate the S1 site is potentially associated with transcriptional control of the osteonectin gene in bone tissue. Deletion mapping and footprinting analysis are underway to delineate other crucial DNA regulatory elements in the osteonectin gene. Genomic clones encoding the PGII gene are also being characterized for similar DNA regulatory elements.

The objective of the Section's bone cell biochemistry program, headed by Dr. Pamela Gehron Robey, is to define bone cell metabolism by investigating specific aspects of cellular activity *in vitro*. The list of bone cell-derived products (which includes type I collagen, osteonectin, osteocalcin, fibronectin, proteoglycan, TGF-ß, and alkaline phosphatase) was expanded by recent studies that definitively identified thrombospondin as a biosynthetic product of osteoblasts. This protein is incorporated into the mineralized matrix of bone and mediates attachment but not spreading of adult human bone cells *in vitro*. Continued study of the biosynthesis of the bone matrix proteins indicates that their rate of synthesis, secretion and compartmentalization between the medium and cell layer/matrix fraction differs among the proteins. It appears that in general, the proteins fall into three classes with respect to rate of synthesis and secretion. A large chondroitin sulfate proteoglycan, CSPG, the small proteoglycan, PG-1, and the heparan sulfate proteoglycan, HSPG, are rapidly synthesized and secreted, whereas collagen, osteonectin, and another small proteoglycan, PG-II, exhibited an intermediate rate, while thrombospondin and fibronectin displayed a relatively slow rate of synthesis and secretion. Detailed analysis of proteoglycan turnover indicates that there are significant differences in the pattern of secretion and turnover rates of all the different bone proteoglycans. Studies utilizing synchronized populations of bone cells also indicate that synthesis and secretion of certain proteins is cell cycle-dependent. Most notably, the expression of alkaline phosphatase drops to virtually undetectable levels during S phase.

Studies on the modulation of osteoblastic metabolism using several factors and hormones have been continued. Interestingly, further investigation using 1,25-dihydroxy vitamin D_3 indicates that while the small proteoglycans, PG-I and PG-II, are highly homologous, synthesis of PG-I is inhibited by this metabolite, while PG-II is unaffected. In further studies on the influence of TGF-ß on bone cells, it was found that unlike our previous data on fetal bovine bone cells, fetal and adult human bone cells are only slightly stimulated by TGF-ß to divide in the presence of 2% fetal bovine serum. However, it should be noted that 2% FBS contains 76 pM TFG-ß, and serum-containing medium is required for adequate human bone cell viability. Human bone cell protein synthesis was decreased approximately 30%, whereas in other connective tissue cells, protein synthesis is generally increased by TGF-ß. In other studies, the effects of fluoride on fetal and adult human bone cells were investigated since fluoride is one of the few therapies that increases bone mass in certain individuals with osteoporosis. It was found that fluoride did not increase cell proliferation rates of adult human bone cells, and slightly inhibited fetal human bone cell growth. In addition, protein synthesis was inhibited by about 25% in both cell systems. These studies indicate that fluoride may not have a direct effect on differentiated osteoblasts, and that the target cell may be an osteoblastic precursor, or another tissue which produces factors that influence bone.

Protein Biophysics Unit

Work during the past year in this Unit, headed by Dr. Dennis A. Torchia, focused upon studies of the structure and dynamics of staphylococcal nuclease in the crystalline state and in solution, with the purpose of attaining a better understanding of the protein structure-function relationship.

Although modern nmr methods have the potential of determining the molecular structure of proteins, the technique has been limited by the difficult problem of assigning the one thousand or more signals observed in the nmr spectra of proteins having molecular weights above 10kD. We have overcome this problem by combining novel two-dimensional nmr spectroscopy with appropriately labeled protein samples, and in this way have assigned signals of nearly the entire backbone and most of the sidechains of S. nuclease complexed with thymidine-3',5'-diphosphate and Ca^{2+}. Nuclease is the largest protein for which such extensive assignments have been obtained.

Combining the information obtained from NOESY spectra and the resonance assignments, we have shown that the three alpha helices in nuclease have regular average structures. Although this result was in apparent disagreement with the crystal conformation deduced from early X-ray diffraction work, a new improved X-ray structure is now in agreement with the nmr result. This shows that nmr is capable of determining solution structures with an accuracy of a few tenths of an angstrom. In addition, nmr measurements of hydrogen exchange rates show that two of the three helices are dynamic structures which periodically open and permit exchange of their hydrogen bonded amide protons with water. The dynamic nature of the nuclease structure was further confirmed by the observation that the aromatic rings of all Phe and Tyr residues, with the sole exception of Y91, execute rapid 180° flips, in spite of the fact that most of these rings are buried within the interior of the protein. The exceptional behavior of Y91 is in accord with the observation that it has the only slowly exchanging hydroxyl proton observed in the protein. We are particularly interested in examining the effect of site mutations at exceptionally stable residues such as Y91 upon the structure, activity and melting temperature of the protein.

Although the protein conformations derived form the nmr and X-ray data are in agreement, at least in so far as most of the protein backbone is concerned, it is highly desirable to compare the protein structure in the crystalline and solution states using the same experimental parameters. We have recently done this for S. nuclease by measuring chemical shifts in the crystalline state (using cross-polarization magic angle spinning) and in solution. Our results show that nine Val amide nitrogen and five Met methyl carbon chemical shifts are nearly identical in the two states. This result shows that the protein adopts nearly the same average conformation in the two states.

Mineral Chemistry and Structure Section

In this Section, headed by Dr. E. David Eanes, membrane-associated acidic phospholipid (APL) molecules have been implicated as playing an important role in regulating mineral deposition in matrix vesicles, the sites of initial calcification in many skeletal tissues. Studies conducted during the year with artifical lipid vesicles (liposomes) suggest that the manner in which these lipids affect the deposition process may, in part, depend upon their relative distribution between the two leaflets of the bilayer membrane. Factors which increased the fraction of APL molecules residing in the inner monolayer of the liposomal membrane increased the time it took for mineral to first appear outside the liposome once intraliposomal mineralization occurred. On the other hand, factors which favored external surface APL locations decreased the time needed to start extraliposomal precipitation but extended the time for its maximum development. Electron microscopic analysis revealed further that mineral crystals became more tightly bound to the liposomal membrane face richest in APL molecules.

These data indicate that interiorly located APL molecules impeded the release of endogenously formed mineral crystals from the liposomes whereas externally situated APL molecules diminished the capacity of these freed crystals to seed new growth, most probably by covering potential growth sites. Since phosphatidylserine, the most abundant APL in matrix vesicles, appears to reside in the inner membrane leaflet, the liposome data suggest that this APL may contribute to vesicle regulation of biocalcification by helping to control the transition from intravesicular to extravesicular mineral formation.

In apatite growth experiments conducted this year in collaboration with members of the American Dental Association Research Group at the National Bureau of Standards, it was found that the rate of apatite precipitation is constant in condensed phase systems where water availability is limited. These data suggest that in hard tissues, where similar limitations are present, new apatite formation is restricted primarily to the boundaries between the mineral-rich zones and the fluid-filled ostoid regions.

Combined previous analyses of a series of hydroxyapatites (HAs) precipitated from aqueous solutions for use as standards and controls indicated their largest impurity is water and that as much as one water molecule per HA unit cell may be structurally incorporated. The α-axis unit-cell dimension of these hydrated HAs (9.423 Å) is larger than that of anhydrous HA (9.418 Å) and it contracts about 0.01Å on thermally removing about one water molecule per HA unit cell by heating from 100 to 500°C; both indicate this water is structurally incorporated. Based on the hexagonal unit-cell contents of HA, there are insufficient chemically analyzed defects or missing ions in these HAs to provide vacant lattice sites to accommodate this structural water component of about one water molecule per unit cell. Consequently, in order to structurally accommodate these excess water molecules, the hexagonal symmetry of the unit cell may be slightly distorted to spatially fit them.

It is known that octacalcium phosphate (OCP) forms double salts with certain aliphatic dicarboxylate anions. In a further collaborative study with M. Markovic and W.E. Brown of the American Dental Association, the formation of OCP-double salts from some structurally different but physiologically relevant polycarboxylates that occur in the Krebs cycle were studied. OCP-double salts were formed with succinate, suberate, adipate, fumarate, and citrate but not with maleate, aspartate or glutamate anions. Nonincorporation of these latter three into OCP may be due to incompatible anion geometry or steric factors. The dominant mechanism of polycarboxylate incorporation in OCP appears to be replacement of HPO_4 ions.

Proteoglycan Chemistry Section

In the Proteoglycan Chemistry Section, headed by Dr. Vincent C. Hascall, the metabolism of hyaluronic acid and proteoglycans was studied in organ cultures of bovine metacarpalphalangeal articular cartilage. The effects of two growth factors, insulin-like growth factor 1 (IGF-1) and transforming growth factor ß (TGF-ß), on these cultures was investigated.

IGF-1 at 10-20 ng/ml as the only medium supplement maintained steady state metabolism of proteoglycans, where synthesis equals catabolism, for at least 6 weeks. This factor increased proteoglycan synthesis (half maximal response at ≈5 ng/ml) and decreased the rate of catabolism (half maximal response ≈1 ng/ml) relative to medium alone and did not promote cell division. TGF ß at 10 ng/ml as the only medium supplement also maintained steady state metabolism of proteoglycans in these cultures. These growth factors, therefore, can be used in a defined medium to study regulation of proteoglycan metabolism without the requirement of serum supplementation, which frequently stimulates cell division and outgrowth and complicates analyses of products released into the medium compartment.

The metabolism of hyaluronic acid in native aggregates was also studied in these cultures and found to be in steady state, with a level of synthesis relative to proteoglycans equivalent to its chemical ratio in the tissue and with the same rate of catabolism as for the proteoglycan. Thus, the mechanisms for regulating proteoglycan catabolism involve entire aggregate structures and probably involve hyaluronate binding proteins at the chondrocyte cell surface.

The synthesis and structure of the keratan sulfate proteoglycan and the dermatan sulfate proteoglycan from corneal stroma (KS-PG and DS-PG, respectively) have been studied using explant cultures of embryonic chicken cornea. Steady-state synthesis of the DS-PG exists in these cultures, but the synthesis of the KS-PG continually declines over the 24 h culture period. Various treatments including pH, medium supplements, and growth factors have not prevented this in vitro decline in stromal KS-PG synthesis. The structures of these two proteoglycan have been characterized with respect to core protein size, GAG composition and size, and oligosaccharride content. Currently, work has begun on peptide mapping these proteoglycans. Monoclonal antibodies have been generated against the KS-PG. These are to be used to immunolocalize this proteoglycan in corneal tissues from various species and developmental age. Also, they will be used to identify the mRNA for the KS-PG core protein and for producing cDNAs for analysis of the amino acid sequence of the core protein. Other studies have been initiated to analyze the proteoglycans in diseased human corneas as well as the proteoglycans in feline corneas after keratoplasty. It is hoped that these studies will reveal information about the role of proteoglycans in the organization of the stromal extracellular matrix in diseases and transplanted cornea.

The presence of haparan sulfate proteoglycan (HSPG) on the cell surface of a wide variety of cells is well established. However, because of the difficulties in purifying these molecules, detailed structural analysis of their core proteins, which is critical for understanding the biological functions of HSPG have not been done. Using proteoglycan purification procedures developed in our laboratory over the years, we have examined HSPG from various rat tissues and analyzed molecular sizes of the core proteins. The results indicate that core proteins of cell surface HSPGs from different cell types are very similar or possibly identical. Detailed structural analyses of core proteins of HSPG will be pursued using a rat osteoblast-like cell line (UMR106), which can provide relatively large quantities of material.

Sources of sulfur contributing to the intracellular PAPS (phosphoadenosine phosphosulfate) pool, the immediate sulfate donor for the sulfation of glycosaminoglycans, have been investigated. In many types of cells, it has been believed that the major sulfate supply to the PAPS pool comes from the extracellular compartments based on the facts that: (1) the intracellular PAPS pool is very small, and (2) when radiolabeled sulfate is added to the culture medium, specific activity of the label in the intracellular PAPS pool rapidly reaches an equilibrium. Contributions of sulfur from cysteine and methionine, the two major possible sulfur sources to the PAPS pool, were measured in rat chondrosarcoma cells and ovarian granulosa cells by analyzing 35S-incorporation into the protein core and glycosaminoglycan moieties of proteoglycans labeled with [35S]cysteine or [35S]methionine. In both cell systems, cysteine contributes less than 1% of the total sulfur to the PAPS pool and methionine contributes less than 0.1% of the total.

Rat parathyroid cells in culture synthesize two distinct species of HSPG. The effects of extracellular calcium, one of the major regulation factors of parathyroid cell function, on HSPG metabolism have been studied. In a normal to high calcium environment, the majority of HSPGs are present in trypsin inaccessible (possibly intracellular) compartments, while in low calcium environments about 50% of the HSPG distribute in the trypsin accessible (cell surface) compartment and translocate rapidly between these two compartments. A change in the extracellular calcium concentration results in the establishment of a new equilibrium in a relatively short time (≤1 hr). Functional roles of HSPG translocation, especially in relation to other molecules which also show similar translocation kinetics in parathyroid cells, and factors which influence such translocation will be further investigated.

Extracellular matrix components of the mouse cumulus cell-oocyte complex (COC) have been studied using metabolic labeling techniques in vitro. Study of this system required development of micro-techniques for analytical procedures, since the amounts of materials to be analyzed are minute. For example, one COC consists of only a few thousand cells. Results indicate that the COC matrix contains large proportions of hyaluronan (≈50% of the total hexosamine) and proteoglycans (≈30%). Stimulation of COC with follicle-stimulating hormone (FSH) results in a dramatic expansion of its matrix, which is believed to be an important step involved in the oocyte development toward fertilization. Analyses of COC matrix labeled with 3H-glucosamine and [35S]-sulfate after treatment with FSH showed dramatic increases in hyaluronan synthesis (≈10 times) and in proteoglycan synthesis (≈5 times), while the synthesis of general glycoproteins increased only 1.5-2 fold. The data suggest that both hyaluronan and proteoglycans play important roles in the process of COC matrix expansion stimulated by FSH.

Bibliography for Bone Research Branch FY88

Arends J, Christoffersen J, Christoffersen MR, Eckert H, Fowler BO, Heughebaert JC, Nancollas GH, Yesinowski JP, Zawacki SJ. A calcium hydroxyapatite precipitated from an aqueous solution-an international multimethod analysis, J Crystal Growth 1987;84:515-532.

Bax A, Sparks SW, Torchia DA. Detection of insensitive nuclei, Advances in Enzymoloyg, In Press, 1988.

Beresford JN, Fedarko NS, Fisher LW, Midura RJ, Yanagishita M, Termine JD, Gehron Robey P. Analysis of the proteoglycans synthesized by human bone cells in vitro, J Biol Chem 1987;262:17164-17172.

Bianco P, Silverstrini G, Termine JD, Bonucci E. Immunohistochemical localization of osteonectin in developing human and calf bone using monoclonal antibodies, Calcif Tiss Intl, In Press, 1988.

Bolander ME, Young MF, Fisher LW, Yamada Y, Termine JD. Osteonectin cDNA sequence reveals potential binding regions for calcium and hydroxyapatite and shows homologies with both a basement membrane protein (SPARC) and a serine proteinase inhibitor (ovomucoid), PNAS 1988;85:2919-2923.

Campbell MA, Handley CJ. The effect of retinoic acid on proteoglycan turnover in bovine articular cartilage cultures, Arch Biochm Biophys 1987;258:143-155.

Campbell MA, Handley CJ, D'Souza SE. Turnover of proteoglycans in articular cartilage cultures. Characterization of medium proteoglycanss, Biochem J 1988, In Press.

Cherney BW, Midura RJ, Caplan AI. Poly (ADP-Ribose) synthetase and chick limb mesenchymal cell idfferentiation, Dev Biol 1985;112:115-125.

Cherney BW, Midura RJ, Caplan AI. Poly (ADP-Ribose) and the differentiation of embryonic tissue, ADP-Ribosylation Reactions 1982;389-405.

Cole HBR, Sparks SW, Torchia DA. A comparison of the solution and crystal structures of staphyloccal nuclease using ^{13}C and ^{15}N chemical shifts as structural fingerprints, PNAS 1988, In Press.

Day AA, McQuillan CI, Termine JD, Young MF. Molecular cloning and sequence analysis of the cDNA for small proteoglycan II of bovine bone, Biochem J 1987;248:801-805.

Eanes ED, Hailer AW, Heywood BR. Modulation of calcium phosphate formation by phosphatidate-containing anionic liposomes, Calcif Tiss Intl, In Press.

Eanes ED. Biophysical aspects of lipid interaction with mineral: Liposome model studies, Anatomical Record, In Press.

Findlay DM, Fisher LW, McQuillan CI, Termine JD, Young MF. Isolation of the osteonectin gene: evidence that a variable region of the osteonectin molecule is encoded within one exon, Biochemistry 1988;27:1483-1489.

Gehron Robey P, Fisher LW, Young MF, Termine JD. The biochemistry of bone, Ostoeporosis 1988;95-109.

Gehron Robey P, Kirshner JA, Cummins III Ce, Ballintine EJ, Rodrigues MM, Gaasterland DE. Synthesis of glycoconjugates by trabecular meshwork of glaucomatous corneoscleral explants, Exp Eye Res 1988;46:111-115.

Gehron Robey P, Termine JD. Biochemical markers of metabolic bone disease, Metabolic Bone Disease 1988, In Press.

Handley CJ, Campbell MA. Catabolism and turnover of proteoglycans, Methods in Enzymology 1987;144:412-419.

Hascall VC. Proteoglycans: The chondroitin sulfate/keratan sulfate proteoglycan of cartilage, ISI Atlas of Science:Biochem, In Press.

Heywood BR, Eanes ED. An ultrastructural study of calcium phosphate formation in multilamellar liposome suspensions, Calcif Tiss Intl 1987;41:192-201.

Hiyama Y, Niu CH, Silverton JV, Bavoso A, Torchia DA, Determination of ^{15}N CSA tensor via ^{2}H-^{15}N dipolar coupling in t-Boc-glycyl-glycyl-(^{15}N)glycyl-benzyl ester, J Am Chem Soc 1988;110:2378-2383.

Luyten FP, Hascall VC, Nissley PS, Morales TI, Reddi AH. Insulin-like growth factors maintain steady state metabolism of proteoglycans in bovine articular cartilage explants, Arch Biochem Biophys 1988, In Press.

Mack JW, Torchia DA, Steinert P. Solid-state NMR studies of the dynamics and structure of mouse keratin intermediate filaments, Biochemistry 1988, In Press.

Midura RJ, Cherney BW, Caplan AI. The relationship of nicotimamide adenine dinucleotide to the chondrogenic differentiation of limb mesenchymal cells, Dev Biol 1985;111:232-242.

Morales TI, Hascall VC. Correlated metabolism of proteoglycans and hyaluronic acid in bovine cartilage organ cultures, J Biol Chem 1988; 263:3632-3638.

Morales TI, Roberts AB. TGFß regulates the metabolism of proteoglycans in bovine cartilage organ cultures, J Biol Chem 1988, In Press.

Morris JE, Yanagishita M, Hascall VC. Proteoglycans synthesized by embryonic chicken retina in culture: Composition and compartmentalization, Arch Biochem Biophys 1987;258:206-218.

NIH LIBRARY

Newsome DA, Hewitt AT, Huh W, Gehron Robey P, Hassell JR. Detection of specific extracellular matrix molecules in drusen, Bruch's membrane, and ciliary body, Am J Ophthalmol 1987;104:373-381.

Newsome DA, Pfeffer BA, Hewitt AY, Gehron Robey P, Hassell JR. Detection of extracellular matrix molecules synthesized in vitro by monkey and human retinal pigment epithelium: influence of donor age and multiple passages, Exp Eye Res 1988;46:305-321.

Sarkar SK, Hiyama Y, Niu CH, Young PE, Gerig JT, Torchia DA. Molecular dynamics of collagen sidechains in hard and soft tissues. A multinuclear magnetic resonance study, Biochemistry 1987;26:6793-6800.

Schulz A, Jundt G, Bergauser KH, Gehron Robey P, Termine JD. Immunohistochemical study of osteonectin in various types of osteosarcoma, Am J Pathol 1988, In Press.

Schwarta R, Young MF, Tsipouras P. Two RFLP's in the 5' end of the human osteonectin gene, Nucl Acids Res 1988, In Press.

Shimokawa H, Ogata Y, Sasaki S, Sobel ME, McQuillan CI, Termine JD, Young MF. Molecular cloning of bovine amelogenin cDNA, Adv Dent Res 1987;1:293-297.

Shishiba Y, Yanagishita M, Hascall VC. Effect of thyroid hormone deficiency on proteoglycan synthesis by human skin fibroblast cultures, Conn Tiss Res 1988;17:119-135.

Shishiba Y, Yanagishita M, Hascall VC, Takeuchi Y, Yokoi N. Characterization of proteoglycans synthesized by rat thyroid cells in culture and their response to thyroid-stimulating hormone, J Biol Chem 1988;263:1745-1754.

Sklenar V, Torchia DA, Bax A. Measurement of carbon-13 longitudinal relaxation using ^{1}H detection, J Magn Reson 1987;73:375-379.

Somerman M, Fisher LW, Foster RA, Sauk JJ. Human bone sialoproteins I and II enhance fibroblast attachment in vitro, Calc Tiss Intl 1988;43:50-53.

Sparks SW, Budhu N, Young PE, Torchia DA. Sidechain dynamics of crystalline L-[3,3-$^2H_2$] methionine studied by deuterium NMR spectroscopy, J Am Chem Soc 1988;110:3359-3367.

Termine JD. Noncollagen proteins in bone. In: Harnett S, Evered D. eds. Cell and molecular biology of hard tissues. Wiley, Chichester, 1988;178-190.

Termine JD. Cellular and molecular biology of bone matrix. In: Christiansen C, Johansen JS, Riis BJ. eds. Norhaven A/S Copenhagen. Osteoporosis 1987;645-652.

Termine JD. Bone proteins, Bone Chem Biochem News Rev 1987;4:15-16.

Termine JD. Biochemistry of the bone matrix. In: Lindh E. eds. Bone and connective tissue markers. New York: Academic Press, 1988, In Press.

Torchia DA, Sparks SW, Bax A. Delineation of α-helical domains in deuterated staphylococcal nuclease by 2D NOE NMR spectroscopy, J Am Chem Soc 1988;110:2320-2321.

Torchia DA, Sparks SW, Bax A. NMR signal assignments of amide proteins in the α-helical domains of S. nuclease, Bichemistry 1988, In Press.

Vogel KG, Koob, JT, Fisher LW. Characterization and interactions of a fragment of the core protein of the small proteoglycans (PGII) from bovine tendon, Biochem Biophys Res Comm 1987;148:658-663.

Wewer UM, Albrechtsen R, Fisher LW, Young MF, Termine JD. Differential deposition of osteonectin in basement membranes of human tissues undergoing profound changes in tissue architecture, Am J Path 1988, In Press.

Young MF, Termine JD. The molecular biology of the bone matrix. In: Christiansen C., Riis BJ eds. Highlights on endocrinology, Copenhagen: Norhaven Bogt, 1987;97-101.

Young MF, Shimokawa HS, Sobel ME, Temrine JD. A characterization of amelogenin messenger RNA in the bovine tooth germ, Adv Dent Res 1987;1:289-292.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00012-26 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fowler, Bruce O.	Research Chemist	BRB NIDR

COOPERATING UNITS *(if any)*

ADAHF, NBS, Gaithersburg, MD
NBS, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish composition and structural details of the apatites in question which include: the type and geometry of constituent ions; the size or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-16 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fisher, Larry W.	Senior Staff Fellow	BRB NIDR
Termine, John D.	Chief, BRB	BRB NIDR
Tuross, Noreen C.	NIH Postdoctoral Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

SIU, School of Dentistry, Edwardsville, IL
Univ. of New Mexico, Albuquerque, NM
FDA, Bethesda, MD

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.35	1.50	1.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The extracellular matrix proteins of the bones and teeth are key elements in the structure and metabolism of these tissues. The goal of this project is to study matrix proteins specific to each mineralizing skeletal tissue in order to understand their molecular structure and biological function.

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binging, RIA, ELISA, etc.) have been developed to quantitate the levels of noncollagenous proteins in bone including osteonectin, bone sialoproteins I and II, bone proteoglycans I and II, and the N-propeptide of type 1(I) collagen (formerly known as 24K phosphoprotein) in (a) surgical specimens of bony tissue and (b) serum (osteonectin). Changes in the noncollagenous protein profile with age and variety of bone (and tooth) diseases have been observed in man and several animal models. We have been highly successful at producing antisera against synthetic peptides for all of the human bone noncollagenous proteins. These antisera have proven useful in immunoprecipitation studies, immunolocalization, immunodetection on Western blots and in the isolation of full length cDNA of human bone osteonectin, proteoglycans I and II, and bone sialoproteins I and II.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-15 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical, Structural and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Eanes, Edward D.	Chief, Mineral Chemistry and Structure Section	BRB NIDR
Heywood, Brigid R.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

American Dental Association Health Foundation, Paffenbarger Research Center, National Bureau of Standards, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.33	1.08	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, healthy bone and teeth. Developmental defects in the deposition of these salts or their destruction and loss by disease can severely impair the function of these skeletal tissues. The purpose of this project is to study the physical, chemical, and ultrastructural properties of these salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable these salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. Topics currently being pursued include (1) the use of artificial lipid vesicles (i.e., liposomes) as in vitro models for investigating the physico-chemical aspects of calcium phosphate precipitate formation in matrix vesicles, and (2) the growth dynamics and size/shape parameters of apatite crystals prepared under physiological-like aqueous solution conditions. The liposome experiments are being conducted with the goal of better understanding how matrix vesicles, the loci for early mineralization in many vertebrate hard tissues, can initiate precipitation in their membrane-bound interior spaces and control the expansion of this initial precipitate into the surrounding extracellular space. The purpose of the crystal growth experiments is to better elucidate the physiochemical and physiological factors delimiting apatite crystal development in various hard tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00134-14 BRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Biosynthesis of Proteoglycans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hascall, Vincent C.	Chief, Proteoglycan Chemistry Section	BRB NIDR
Morales, Teresa I.	Senior Staff Fellow	BRB NIDR
Midura, Ronald	NIH Postdoctoral Fellow	BRB NIDR
Campbell, Margaret	Visiting Fellow	BRB NIDR
Calabro, Anthony	Guest Worker	BRB NIDR
Chang, Eric	Chemist	BRB NIDR

COOPERATING UNITS *(if any)*
Rush-Presbyterian-St. Luke's Medical Center, Univ. of Lund, Sweden
University of West Virginia
University of Michigan

LAB/BRANCH
Bone Research Branch

SECTION
Proteoglycan Chemistry Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.09	4.59	.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to study the chemical and physical properties and biosynthesis of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the core protein of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of core protein precursors and processing to mature proteoglycans; 3) Effects of bacterial endotoxins (lipopolysaccharides), interleukin-1 and growth factors (insulin-like growth factors and transforming growth factor beta) on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilages; 4) The regulation of hyaluronic acid synthesis and catabolism in organ cultures of bovine articular cartilage; 5) sulfate-proteoglycan and dermatan sulfate-proteoglycan in chick, cat, and human cornea.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00157-13 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies on the Structure of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Torchia, Dennis A.	Chief, Protein Biophysics Unit	BRB NIDR
Sparks, Steven W.	Staff Fellow	BRB NIDR
Cole, Holly	Staff Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

York College, SUNY, Jamaica, NY; DB, NCI, NIH; LC, NHLBI, NIH
University of Maryland; LB, NCI, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Unit

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	3.00	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to investigate the molecular structure and dynamics of proteins and model compounds. The structural and dynamical information obtained will be correlated with function. Areas of present interest are 1) Calcium binding proteins. We are using multinuclear nmr to study (a) the molecular dynamics (b) the structure and (c) the interactions of staphylococcal nuclease with calcium, and with inhibitors and model substrates, 2) We are continuing our program of detailed studies of molecular dynamics of various small molecules which serve as dynamic models of proteins. 3) We are setting up nmr facilities to study the structure, dynamics and functions of HIV related proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00379-05 BRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Bone Matrix Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Young, Marian F.	Senior Staff Fellow	BRB NIDR
Termine, John D.	Chief	BRB NIDR
Day, Agnes A.	Staff Fellow	BRB NIDR
Findlay, David M.	Visiting Fellow	BRB NIDR
Dominguez, Pedro	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.93	3.43	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The matrix proteins of bones play key roles in the structure and function of these tissues. Our objective in this investigation is to study the biosynthesis of these macromolecules and to understand the regulation of their expression.

The expression of bone matrix proteins have been studied by constructing recombinant cDNA libraries from bone cell mRNA. cDNA clones encoding several bone and tooth matrix proteins have been isolated using expression DNA vectors and polyclonal antisera directed against individual bone matrix and ameloblast proteins. The clones were used to study the primary structure and regulation of expression of these genes in cultured bone cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00380-05 BRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Bone Cells in Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gehron Robey, Pamela	Biologist	BRB NIDR
Termine, John D.	Chief	BRB NIDR
Fedarko, Neil S.	IRTA	BRB NIDR
Kopp, Jeffrey B.	Staff Fellow	BRB NIDR
Heywood, Brigid R.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Bone Research Branch

SECTION
Skeletal Biology

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	3.45	1.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bone cells derived from a variety of animal species including human, bovine, ovine and rodent, and of varying developmental ages, have been utilized to:
1) study the biosynthesis and deposition of extracellular matrix proteins such as collagen, osteonectin, bone proteoglycan and other bone proteins, and alterations of matrix production in the disease Osteogenesis Imperfecta;
2) study the responsiveness of the cells to a variety of hormonal and pharacoloical factors (such as 1,25-dihydroxy vitamin D3 and fluoride);
3) elucidate the production and interaction of growth factors (such as TGF-β, insulin-like growth factors and platelet-derived growth factors);
4) study the potential function of bone matrix proteins through the use of functional assays and the use of anti-sense DNA to inhibit specific protein synthesis;
5) serve as a source of mRNA and DNA for studies of the proteins at the genomic level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00431-02 BRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Proteoglycans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yanagishita, Masaki	Visiting Scientist	BRB NIDR
McQuillan, David J.	Visiting Associate	BRB NIDR
Salustri, Antonietta	Visiting Associate	BRB NIDR
Sayed, Atef K.	Senior Staff Fellow	BRB NIDR
Hascall, Vincent C.	Chief, Proteoglycan Chemistry Sect.	BRB NIDR

COOPERATING UNITS *(if any)*
Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL; HGB, NICHD, NIH
Toranomon Hospital, Tokyo, Japan; NIADDK, NIH; Univ. of West Virginia;
University of Maryland

LAB/BRANCH
Bone Research Branch

SECTION
Proteoglycan Chemistry Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.33	4.08	.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to study biochemical and physical properties, biological function and metabolism of proteoglycan under physiological and various pathological conditions using a number of tissues and cell systems. Topics of present interest include: (1) Metabolism of sulfur containing amino acids as sulfate sources in chondrosarcoma and granulosa cell proteoglycan biosynthesis, (2) analysis of proteoglycan structure in a osteoblast-like cell line, (3) analysis of proteoglycans in a parathyroid cell, and (4) analysis of proteoglycans and hyaluronic acid in mouse cumulus cell-oocyte complex.

ANNUAL REPORT OF THE LABORATORY OF CELLULAR DEVELOPMENT AND ONCOLOGY

NATIONAL INSTITUTE OF DENTAL RESEARCH

The reorganization of the Laboratory, planned and partially implemented during the years 1985-1987, is, as yet, not completed. Dr. John Folk, Chief of the Enzyme Chemistry Section, continues to serve as the acting chief. Organizational changes in the Laboratory were followed by a formal request to change the name of the Laboratory and to create a new section and a new unit. This request has been granted and the Laboratory of Cellular Development and Oncology, formally the Laboratory of Oral Biology and Physiology, is now composed of three Sections and one Unit. The new Section, the Molecular and Cellular Biology Section, the chief of which will not be officially transferred from the National Cancer Institute until laboratory renovations are completed early next year, is of similar size and composition to the Enzyme Chemistry and Bone Cell Biology Sections. The Peptide and Immunochemistry Unit, one function of which continues to be to provide custom made peptides to all extramural programs, is of smaller size. Laboratory personnel total about 25 with a ratio of research to support staff of about 5:1. Within the research staff the ratio of temporary (postdoctoral fellows and visitors) to tenure staff is also about 5:1. Of the temporary scientists only one-third are supported by mechanisms that do not count against our position ceiling. The size of the laboratory programs is thus largely limited by this ceiling and our ability to recruit through other mechanisms. Programs size, however, should in practice also be measured to include collaborations with scientists elsewhere in NIDR, NIH, and outside of NIH. All of our scientific staff members collaborate closely with scientists outside of the laboratory and in this way promote mutual productivity.

In the early spring of this year, the laboratory was reviewed by the NIDR Board of Scientific Counselors. Encouragement of the laboratory staff was provided by the Board's uniform rating of excellent for research accomplishments.

The research achievements of the Laboratory detailed below were made possible by the diverse expertise of members of the staff, the numerous collaborations with scientists outside of the Laboratory, the development of novel and powerful technologies and their applications to the programs, and the interdisciplinary nature of the Laboratory.

Bone Cell Biology Section

A challenging problem in bone cell biology is the control of bone formation and repair by growth and differentiation factors in the extracellular matrix. The bone morphogenetic factor osteogenin was further purified (up to 220,000-fold total) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroelution. It appears to be a glycoprotein of molecular weight about 30,000, which is not active alone *in vivo*, but for assay must be combined with type

I collagen from bone matrix before implantation intramuscularly in the rat.

The presence of transforming growth factor-β (TGFβ) in bone matrix was studied using the ossicle model. During bone development TGFβ starts to accumulate at a time when ossification during bone development takes place. It appears that the cell types that, in vivo, could contribute to the high TGFβ content of bone matrix are polymorphonuclear leukocytes and osteoblasts, chondrocytes not withstanding.

The multifunctional protein that regulates proliferation and differentiation of a variety of mesodermal and neuroectodermal cells, fibroblast growth factor (FGF), exists in both acidic and basic forms. The role of its forms in cartilage and bone development was investigated by a study of gene expression using cDNA probes and assay of FGF by a radioimmunoassay procedure. Basic FGF alone and together with platelet-derived growth factor did not initiate bone formation, whereas inhibition of bone formation by locally implanted demineralized bone matrix was induced by as little as 0.5 to 1 μg of basic FGF.

Study of the influences of insulin-like growth factors I and II (IGF-I and IGF-II, respectively) on the biosynthesis and catabolism of bovine cartilage proteoglycans in implants indicated that chondrocytes regulate proteoglycan synthesis. This control is primarily via the IGF-I receptor. The smaller IGF-II response is also through this same receptor. Preliminary evidence was obtained for a chondrocyte differentiation factor in cartilage tissue, distinct from osteogenin.

A partially purified osteogenin preparation was found to induce healing in an experimentally produced craniofacial defect in the rat. This surgically formed defect, which would normally not heal during the life of the animal, completely healed in less than three weeks upon osteogenin treatment.

Peptide and Immunochemistry Unit

HIV-I is the causative agent of AIDS. A synthetic 22-member peptide analogous to a region of the CD-4 receptor molecule from infectable cells was found to block binding of HIV-I and its envelope protein gp-120 to CD-4, to bind to gp-120 itself, and to inhibit syncitia formation in vitro. A polymerized form of this peptide was found to be far more potent in these respects. Attempts are underway to utilize this information to design special drug delivery systems and to prepare vaccines.

The chemistry needed for facile and efficient peptide polymerization in the preparation of synthetic vaccines has been under intensive study. By automated proper insertion of -SH and bromoacetyl or chloroacetyl groups into peptides during their synthesis, it has been possible to attain polymerization to give products in excess of

20,000 molecular weight. These products should circumvent the need for protein carriers in antibody formation.

Two human serum proteins, C-reactive protein (CRP) and serum amyloid P component (SAP), are closely related in their primary structures. It is possible, because of this close relationship in structure, that these two proteins act in concert during acute phase response. The exact function of SAP is not known, however. A synthetic peptide modeled after a portion of the SAP sequence was found to support cell attachment in a model system, whereas SAP itself has no such activity. It is possible that the cell attachment peptide is produced from SAP under physiological conditions and a search for a possible mechanism of its production is underway. There is also indication that this peptide may induce chemotaxis of cells that synthesize and express fibronectin.

Enzyme Chemistry Section

Eukaryotic protein synthesis initiation factor 4D (eIF-4D) is the only cellular protein that contains the amino acid hypusine. The ubiquity of hypusine in mammalian cells, its occurrence in a single protein, that it occurs at a single position in this protein, and the complex mechanism of its posttranslational biosynthesis have combined to stimulate exploration of the manner and control of its formation and of its physiological function.

Use was made of difluoromethylornithine (DFMO), an inhibitor of cellular polyamine metabolism, to promote accumulation of an eIF-4D precursor in CHO cells. This protein was demonstrated to be one in which the lysine residue was not converted to the hypusine precursor, deoxyhypusine. Careful study of the structure of this protein also revealed that there are posttranslational steps in the biogenesis of eIF-4D in addition to the one involving formation of deoxyhypusine *per se*. Control of these first steps in hypusine biosynthesis by cellular spermidine levels was evident from this study.

Cell-free synthesis of deoxyhypusine was achieved with the use of lysates of DFMO-treated cells. The substrate, eIF-4D precursor, and the enzyme, deoxyhypusine synthetase, were separated and purified. Using the purified system, 1,3-diaminopropane was identified as the cleavage product of spermidine formed in conjunction with butylamine transfer to form deoxyhypusine.

Further studies on deoxyhypusine hydroxylase, the enzyme that catalyzes conversion of deoxyhypusine to hypusine, showed it to be inhibited by polyamines, suggesting a degree of regulation also in this final step of eIF-4D formation. This observation is in accordance with earlier evidence for a correlation between deoxyhypusine hydroxylase activity and cellular proliferation. Inhibition of deoxyhypusine hydroxylase by deoxyhypusine-containing synthetic peptides and by metal chelator-linked synthetic peptides paved the way to further studies on the enzyme mechanism and to the design of mechanism-based inhibitors for use in cellular studies.

The transglutaminases catalyze a posttranslational transamidation reaction that results in crosslink formation between protein molecules through γ-glutamylamide bonds. Although the catalytic properties and mechanism of this group of enzymes were under intensive study for a number of years by members of the Enzyme Chemistry Section, only recently was it recognized that these enzymes, through their directed specificity, provide important biological regulatory functions. Their roles in biological control are in addition to their known purpose of providing structural integrity and extensibility limitations through crosslink production between protein molecules.

Osteonectin, a non-collagenous, bone, plasma, and platelet glycoprotein with high affinity for type I collagen, thrombospondin, and hydroxyapatite, was observed to covalently crosslink to fibrin during the clotting of fibrinogen. This crosslinking, which is transglutaminase catalyzed, may initiate matrix formation important in wound healing and/or normal growth.

The ability of tumors to activate the hemostatic system contributes to their invasive and metastatic behavior. It was observed that murine melanoma B16/F10 cells, which are effectively lysed by murine lymphokine-activated killer (LAK) cells, are protected against this lysis by addition of fibrinogen to the medium. This resistance of B16/F10 cells to LAK cells results from covalent attachment of fibrin a-chains to B16/F10 cell membrane proteins and thus to a cocooning of the cells in a protective fibrin layer. The reaction appears to be transglutaminase catalyzed and provides a model for a mechanism by which tumor cells may survive during the dissemination process.

The mature cell envelope of the stratum corneum is formed beneath the plasma membrane during terminal differentiation of epidermal cells. This envelope, which is vitally important for the resistance of skin to external insults, is composed of several cellular proteins covalently connected by transglutaminase-catalyzed crosslinking. The ε-(γ-glutamyl)lysine bond was known for a number of years to be the connecting link. This year we discovered another transglutaminase-catalyzed crosslink in cell envelope from normal skin. This is a polyamine-containing bond, bis(γ-glutamyl)spermidine, which is present at levels comparable to those of the ε-(γ-glutamyl)lysine bond. Significantly higher levels of both of these crosslinks were observed in envelopes from involved areas (scales) of psoriatic patients, as compared with those from unafflicted areas (callus) of these patients and those from callus of normal individuals. These findings point up a direct involvement of a polyamine metabolic aberration in psoriasis and suggest that cell envelope crosslink disorders may occur in other dermatological conditions in which there are stratum corneum abnormalities.

This brief review shows that the Laboratory has been active and productive in all of its programs with new results leading to new projects. With the official change in the name of the Laboratory and the creation of two new units, all that remains to be completed are the room renovations needed to house the incoming Molecular and Cellular Biology Section. Emphasis has been on growth and cellular regulation. Even more intense emphasis on these vital topics is expected with the advent of a functioning program on malignant disease.

Laboratory of Cellular Development and Oncology
PUBLICATIONS

Abbruzzese A, Liguori V, Isernia T, Park MH. Inhibition of Deoxyhypusine Hydroxylase by Polyamines. Italian J. Biochem (in press).

Beninati S, Martinet N, Folk JE. High Performance Liquid Chromatographic Method for the Determination of ε(γ-Glutamyl)lysine and mono- and Bis-γ-glutamyl Derivatives of Putrescine and Spermidine. J. Chromat. 1988;443:329-335.

Beninati S, Piacentini M, Cocuzzi ET, Autuori F, Folk JE. Covalent Incorporation of Polyamines as γ-Glutamyl Derivatives in CHO Cell Protein. Biochem. Biophys. Acta. 1988;952:325-333.

Bleiberg I, Ricciardone MD, Reddi AH, McCarthy KF. New bone formation and bone marrow differentiation induced in rats by extracellular bone matrix implantation. Effect of local preirradiation on the process. Exp. Hematol. 1987;15:309-315.

Burns DL, Hausman SZ, Lindner W, Robey FA, Manclark, CR. Structural characterization of pertussis toxin A subunit. J. Biol. Chem. 1987;262:17677-17682.

Carrington JL, Roberts AB, Flanders KC, Roche NS, Reddi AH. Changes in the accumulation, localization and expression of message for transforming growth factor β during endochondral bone and marrow development. J. Cell Biol. (in press) .

Chung SI, Chang SK, Cocuzzi E, Folk JE, Kim HC, Lee SY, Martinet N, Nigra T, Sun HS. Modulation of Cellular Transglutaminase: protease-induced activation. Advances in Experimental Medicine and Biology 1988;231:1-14.

Golding H, Robey FA, Gates FT III, Lindner W, Hoffman T, Golding B. Identification of homologous regions in HIV 1 gp41 and human MHC Class II beta 1 domain. I. Antibodies against the gp41 derived peptide react with native HLA Class II antigens suggesting a role for autoimmunity in the pathogenesis of AIDS. J. Exp. Med. 1988; 167:914-923.

Graf J, Ogle RC, Robey FA, Sasaki M, Martin GR, Yamada Y, Kleinman HK. A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. Biochem. 1987;26:6896-6900.

Habig WH, Halpern JL, Groover KA, Lindner W, Robey FA. Synthetic peptide of tetanus toxin: Use as immunogens and structureal probes. In: Eighth International Conference on Tetanus 1988, in press.

Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y,

Martin GR. YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. Science 1987;238:1132-1134.

Iwamoto Y, Graf J, Sasaki M, Kleinman HK, Martin GR, Robey FA, Yamada Y. A synthetic peptide from the B1 chain of laminin is chemotactic for B16F10 melanoma cells. J. Cell. Physiol. 1988;134:287-291.

Kinchington PR, Inchuaspe G, Subak-Sharpe JH, Robey F, Straus SE, Hay J, Ruyechan WT. Identification and characterization of a Varicella Zoster virus DNA-binding protein using antisera directed against a synthetic peptide. J. Virol. 1988;62:802-809.

Lee KN, Chung SI, Girard JE, Fesus L. Evaluation of phenylthiourea derivatives as inhibitors of transglutaminase-catalyzed reaction in chinese hamster ovary cells. Biochim Biophys Acta (in press).

Luyten FP, Hascall VC, Nissley SP, Morales TI, Reddi AH. Insulin-like growth factors maintain steady state metabolism of proteoglycans in bovine articular cartilage explants. Arch. Biochem. Biophys. (in press).

Maor G, von der Mark K, Reddi AH, Heinegard D, Franzen, A, Silbermann M. Acceleration of cartilage and bone differentiation on collagenous substrata. Coll. Rel. Res. 1987;7:351-370.

Martinet N, Kim HC, Girard JE, Nigra TP, Strong DM, Chung SI, Folk JE. Epidermal and Hair Follicle Transglutaminases. Partial Characterization of the Soluble Enzymes in Newborn Mouse Skin. J. Biol. Chem. 1988;263:4236-4241.

Muthukumaran N, Ma S, Reddi AH. Dose-dependence of and threshold for optimal bone induction by collagenous bone matrix and osteogenin-enriched fraction. Coll. Rel. Res. (in press).

Park MH. Regulation of Hypusine in Chinese Hamster Ovary Cell. J. Biol. Chem. 1987;262:12730-12734.

Park MH. Identification of an Eukaryotic Initiation factor 4D precursor in spermidine-depleted Chinese Hamster Ovary cells. J. Biol. Chem. 1988;263:7447-7449.

Park MH, Abbruzzese A, Folk JE. Posttranslational Formation of Hypusine: Biogenesis of Translational Initiation Factor eIF-4D. In: Advances in Posttranslational Modification of Proteins and Ageing 1988:633-640.

Park MH, Wolff EC. Cell-Free Synthesis of Deoxyhypusine Separation of Protein Substrate and Enzyme, and Identification of 1,3-diaminopropane as a product of spermidine cleavage J. Biol. Chem. (in press)

Park MH, Wolff EC, Abbruzzese A, Folk JE. Biosynthesis of Hypusine in eIF-4D precursor. Advances in Polyamine Research (in press).

Piacentini M, Martinet N, Beninati S, Folk JE. Free and Protein-Conjugated Polyamines in Mouse Epidermal Cells. Effect of High Calcium and Retinoic Acid. J. Biol. Chem. 1988;263:3790-3794.

Reddi AH, Wientroub S, Muthukumaran N. Biologic principles of bone induction. Orthop. Clinics of North America 1987;18:207-212.

Robey PG, Young MF, Flanders KC, Roche NS, Kondaiah P, Reddi AH, Termine JD, Sporn MB, Roberts A. Osteoblasts synthesize and respond to transforming growth factor-type β (TGF-β) in vitro. J. Cell Biol. 1987;261:5693-5695.

Roth BL, Mehegan JP, Jacobowitz D, Robey FA, Iadorola MJ. Rat protein kinase C: Purification, antibody production and quantification in discrete regions of hippocampus. J. Neurochem. 1988 (in press).

Roth WJ, Chung SI, Raju L, Janoff A. Characterization of two distinct transglutaminases of murine bone marrow-derived macrophages: effects of exposure of viable cells to cigarette smoke on enzyme activity. J. Leukocyte Biology 1988;42:9-20.

Sampath TK, Muthukumaran N, Reddi AH. Isolation of osteogenin and extracellular-matrix associated bone inductive protein by heparin affinity chromatography. Proc. Nat. Acad Sci.USA 1987;84:7109-7113.

Sedegah M, Beaudoin RL, De la Vega P, Leef MF, Oscel A, Jones E, Charoenvit Y, Yuan LF, Gross M, Majarian WR, Robey FA, Weiss W, Hoffman SL. Use of a vaccinia contruct expressing the circumsporozite protein in the analysis of protective immunity to plasmodium yoelii. In: Technological advances in vaccine development. UCLA Symposium on Molecular and Cellular Development, New Series, Vol. 84, (Laskey, L., ed.) Alan, R. Liss, Inc. New York, N.Y. (1988) (in press).

Silbermann N, Reddi AH, Hand AR, Leapman RD, von der Mark K, Franzen A. Chondroid bone arises from mesenchymal stem cells in organ culture of mandibular condyles. J. Craniofacial Genetics Dev. Biol. 1987;7:59-79.

Silbermann M, Reddi AH, Hand AR, Leapman RD, von der Mark K, Franzen A. Further characterization of the extracellular matrix in the mandibular condyle in neonatal mice. J. Anat. 1987;151:169-188.

Silbermann M, Tenenbaum H, Livne E, Leapman R, von der Mark K, Reddi AH. The *in vitro* behavior of fetal condylar cartilage in serum-free hormone supplemented medium. Bone 1987;8:117-126.

Wientroub S, Price, PA, Reddi AH. The dichotomy in the effects of 1,25 dihydroxyvitamin D_3 and 24,25 dihydroxyvitamin D_3 on bone α-carbodyglutamic acid-containing protein in serum and bone in vitamin-D deficient rats. Calcif. Tiss. Int. 1987;40:166-172

Wientroub S, Reddi AH. Influence of irradiation on the osteoinductive potential of demineralized bone matrix. Calcif. Tiss. Int. (in press).

Williams WV, London SD, Weiner DB, Wadsworth S, Berzofsky JA, Robey FA, Rubin DH, Greene MI. Immune response to a molecularly defined internal image idiotype. J. Exp. Med. 1988, (in press).

Wlodarski K, Reddi AH. Tumor cells stimulate *in vivo* periosteal bone formation. Bone and Mineral 1987;2:185-192.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00001-36 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transglutaminases: Functions, Control and Biological Roles of Products.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI:	Folk, J.E.	Acting Chief	LCDO NIDR
OTHERS:	Martinet, N.	Guest Researcher	LCDO NIDR
	Beninati, S.	Visiting Associate	LCDO NIDR
	Chen, G.	Guest Researcher	LCDO NIDR

COOPERATING UNITS *(if any)*

Dr. M. Piacentini, 2nd University, Rome, Italy; Dr. J. Gorman, CSIRO, Australian National Health Laboratory, Geelong, Australia; Dr. M. Fink, Baylor University, Waco, Texas.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.56	1.58	.98

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the basis for differences in specificities of the transglutaminases are underway. Specific inhibitors for these enzymes have been prepared. Several intracellular products of transglutaminase action were identified. The catabolic fate of the transglutaminase products is now understood.

Investigators:

Folk, J.E.	Acting Chief	LOBP NIDR
Martinet, N.	Guest Researcher	LOBP NIDR
Beninati, S.	Visiting Associate	LOBP NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00049-17 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chung, S.I.	Research Chemist	LCDO NIDR
OTHERS:	Cardinali, M.	Visiting Fellow	LCDO NIDR
	Kanemitsu, K.	Visiting Fellow	LCDO NIDR
	Kim, H.C.	Guest Researcher	LCDO NIDR
	Termine, J.E.	Chief, BRB	BRB NIDR
	Fisher, L.W.	Senior Staff Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

Dr. Sang Chul Park, Seoul National University, Seoul, Korea.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.00	3.75	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of "provisional stromata" (fibrin or fibrin-connective tissue), during tissue or bone fracture repair, and in the modulation of specific cellular processes. The coagulant layer formed at injury sites is one of the vital elements of hemostasis and diathesis. The major constituent of the coagulant gel is fibrin. Fibrin stability appears to dictate overall healing and restoration processes and is modulated by factor XIIIa-catalyzed cross-linking of fibrin subunits and of alpha-2-antiplasmin to alpha-chain of fibrin. A number of other plasma proteins are also known to cross-link to fibrin, e.g., fibronectin, thrombospondin, and von Wilerbrand factor. Osteonectin, a non-collagenous bone glycoprotein which exhibits high affinity for type I collagen, thrombospondin, and hydroxyapatite was shown to cross-link to fibrin and also was found to be a substrate for cellular transglutaminase. Fibrin clots provide matrices for the initial phase of cell migration and anchorage, and have been found to be covalently cross-linked to cell membrane, e.g., in B16 melanoma cells. The fibrin cross-linked to surface of tumor cells provides a protective shield for melanoma cells against LAK cell-induced cell-lysis. Transglutaminase in terminally differentiated epidermis catalyzes the cross-linking of cellular proteins to form stabilized cornified envelope. One of the enzymes which represent the majority of cytosol enzyme activity was found to be present in inactive form and was activated by either high calcium or by proteases.

PHS 6040 (Rev. 1/84)

GPO 914-918

Investigators:

Chung, S.I.	Research Chemist	LCDO NIDR
Cardinali, M.	Visiting Fellow	LCDO NIDR
Kanemitsu, K.	Visiting Fellow	LCDO NIDR
Kim, H.C.	Guest Researcher	LCDO NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00204-12 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth and Differentiation Factors in Bone and Cartilage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reddi, A.H.	Chief, BCBS	LCDO NIDR
OTHERS:	Carrington, J.	IRTA Fellow	LCDO NIDR
	Cunningham, N.	Research Chemist	LCDO NIDR
	Harrison, E.	Research Biochemist	LCDO NIDR
	Luyten, F.	Visiting Associate	LCDO NIDR
	Ma, S.	Visiting Fellow	LCDO NIDR
	Muthukumaran, N.	Guest Researcher	LCDO NIDR
	Katz, R.	Dental Staff Fellow	CIPCB NIDR

COOPERATING UNITS *(if any)*

Dr. J. Hollinger, U.S. Army Institute of Dental research, Washington, DC; Dr. A. Roberts, NCI; Dr. P. Nissley, NCI.

LAB/BRANCH

Laboratory of Cellular Development and Oncology.

SECTION

Bone Cell Biology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7.35	6.0	1.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to investigate the growth and differentiation factors in bone and cartilage. The research projects under investigation are: 1) osteogenins from bone matrix; 2) changes in the expression, localization and compartmentation of transforming growth factor β in developing endochondral bone; 3) role of fibroblast growth factor in bone development; 4) actions of insulin-like growth factors on cartilage, and 5) application of osteogenin in correction of craniofacial defects.

Investigators:

Reddi, A.H.	Chief, BCBS	LCDO, NIDR
Carrington, J.	IRTA Fellow	LCDO, NIDR
Cunningham, N.	Research Chemist	LCDO, NIDR
Harrison, E.	Research Biologist	LCDO, NIDR
Luyten, F.	Visiting Associate	LCDO, NIDR
Ma, S.	Visiting Fellow	LCDO, NIDR
Muthakumaran, N.	Dental Staff Fellow	LCDO, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00311-08 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Translation Initiation Factor 4D; Structure, Biosynthesis and Control.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Park, M.H.	Senior Staff Fellow	LCDO NIDR
OTHERS:	Folk, J.E.	Acting Chief	LCDO NIDR
	Wolff, E.C.	Chemist	LCDO NIDR

COOPERATING UNITS *(if any)*

Dr. Hanauske-Abel, Harvard Medical School, Boston, MA; Dr. A. Abbruzzese, 1st Medical School, University of Naples, Naples, Italy.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.85	2.50	1.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eukaryotic protein translation initiation factor 4D (eIF-4D) contains one residue of hypusine and appears to be the only cellular protein with this one unique amino acid. Hypusine is produced posttranslationally by transfer of the butylamine portion of the polyamine spermidine to a lysine residue in the eIF-4D precursor and subsequent hydroxylation. These findings reveal a novel cellular metabolic pathway. Studies are underway to relate the structure of hypusine to the function of eIF-4D and to understand the posttranslational control of hypusine biosynthesis.

Folk, J.E.	Acting Chief,	NIDR
Park, M.H.	Senior Staff Fellow	NIDR
Wolff, E.C.	Chemist	NIDR
Gould, L.M.	Biological Lab Technician	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00433-02 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Aspects of C-Reactive Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Research Chemist	LCDO NIDR
OTHERS:	Dhawan, S.	Senior Staff Fellow	LCDO NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two serum proteins that are closely related in their primary structures are C-reactive protein (CRP) and serum amyloid P component (SAP). Perhaps the key difference between the two are their modes of biosynthesis; whereas SAP is a normal serum protein with constant levels in the serum regardless of the disease state of the person, the serum levels of CRP raise rapidly with the onset of inflammation. The function of CRP is now believed to mediate the removal of chromatin that is present in the cellular debris following cell death. The function of SAP is unknown.

We have found in the primary structure of SAP a peptide, YIGR, which resembles the cell attachment peptide and was tested for cell attachment activity. In contrast to our expectations, a control peptide, 13 amino acids in length, was found to have strong cell attachment activity. The peptide containing YIGR was inactive. Cell attachment studies have been conducted on the cell attachment peptide from SAP and further studies have shown that the SAP peptide is binding to fibronectin. Thus, we have identified a unique fibronectin-binding peptide which may be useful for acting as a substrate to attach cells to insoluble matrices.

The significance of the research is that we have identified a new cell attachment peptide and formation of this peptide by proteolysis of the native protein may be a first step in repair of damaged tissue.

Investigators:

Robey, F.A.	Research Chemist	LCDO NIDR
Dhawan, S.	Senior Staff Fellow	LCDO NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00434-02 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis and Use of Peptides from HIV-1 Having Homology to Host Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Research Chemist	LCDO NIDR
OTHERS:	Nahor, O.	Visiting Fellow	LCDO NIDR
	WAhl, S.	Microbiologist	LMI NIDR

COOPERATING UNITS *(if any)*

M. Roberts-Guroff, NCI.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.90	.65	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HIV-1 is the causative agent of AIDS. To date there are no promising vaccines or drugs to combat HIV-1. Our approach to the AIDS problem is to use peptide chemistry to develop potential vaccine candidates and new drugs. The work is based on the fact that CD-4 is the cellular receptor for the virus and that the amino acid sequence of CD-4 is well-known. In addition, we are relying on other published data to guide us in determining what the ligand for CD-4 on the virus is. Using this as background information our goal is to design and synthesize reagents which are capable of blocking the binding of the virus to cells. Next we will conjugate anti-viral drugs to these reagents and begin tests to evaluate their in vitro efficacy.

The primary focus of our work is to develop a "guided missle" anti-HIV-1 reagent. Such a reagent will be able to recognize the receptor for the virus, bind to the receptor and enter the cell through the receptor. In addition, our goal is to not damage the cell but to kill the virus. We are currently developing monoclonal antibodies directed against CD-4, the HIV-1 receptor. Antibodies are against peptides derived from the receptor and react with the native receptor protein. This makes them unique from what is commercially available. Studies are currently underway to evaluate which anti CD-4 monoclonals are taken up by CD-4 expressing cells.

The location on the viral envelope glycoprotein which binds to CD-4 is now known for some isolates of the virus. Peptide polymers of these are being synthesized for use in raising antibodies. Vaccine efficacy will be determined from these studies. In addition to the monoclonal antibody work mentioned above, we are trying to determine the specificity of certain CD-4 binding peptides as potential drug-carrying reagents.

Investigators:

Robey, F.A.	Research Chemist	LCDO NIDR
Nahor, O.	Visiting Fellow	LCDO NIDR
Wahl. S.	Microbiologist	LMI NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00437-02 LCDO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peptide Polymers as Vaccine Candidates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Research Chemist	LCDO NIDR
OTHERS:	Dhawan, S.	Senior Staff Fellow	LCDO NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.20	.45	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a few isolated instances researchers have found that synthetic peptides act as suitable immunogens to provide protection against viruses. In order to function in this manner, a peptide of interest must be coupled to a carrier protein in order that it remain in the host long enough for a immune response to develop. We are trying to improve on the current peptide-carrier protein conjugation strategy by developing vaccines composed of peptide polymers. Theoretically, a high molecular weight polymer of a peptide should be immunogenic. These types of polymers may constitute a new generation of vaccine candidates because there would no longer be a need for the carrier proteins. Such vaccines of highly defined chemical composition may be specific for a certain pathogen, without the side effects that often occur using the peptide-carrier protein approach.

A. Peptide synthesis - Various test peptides are synthesized and polymerized for immunization purposes. Parallel to this the same peptides are coupled to carrier proteins, such as bovine serum albumin.

B. Immunization studies - Rabbits are immunized with peptide polymers or peptide carrier protein conjugates and the antibody responses are evaluated using enzyme linked immunosorbant assays.

C. New coupling chemistry - We have developed a method to automatically place a bromoacetyl moiety at the amino termini of synthetic peptides. Advantages of this method over the chloroacetyl method are being evaluated.

Investigators:

Robey, F.A.	Research Chemist	LCDO NIDR
Dhawan, S.	Senior Staff Fellow	LCDO NIDR

NIH LIBRARY

ANNUAL REPORT OF THE CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH NATIONAL INSTITUTE OF DENTAL RESEARCH

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinical and basic research programs; (2) to offer consultation on oral and dental problems to other institutes and render clinical care to specified patients; (3) to encourage and facilitate clinical research activities of other branches and laboratories within the Institute; and (4) to sponsor an oral medicine training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research-oriented dental clinicians.

The past year has seen remarkable progress in our laboratory research program with our overall effort directed at studying the regulation of glandular epithelial cell secretory processes, (eg. ion fluxes and fluid movement, gene expression, protein routing and release). Likewise our strong center of clinical research has been expanded. We now have major substantive efforts in four areas; dry mouth/xerostomia and salivary hypofunction, diagnostic and management problems posed by specific compromised patient groups (oncology, congenital disorders); aging and oral physiology; and oral manifestations of AIDS. Furthermore collaborative interactions with other laboratories or branches, both within and outside NIDR, have continued to increase. There is an extremely high level of effort, cooperation, flexibility and understanding by Branch personnel which has allowed us to make great strides towards reaching our overall goals.

NIH LIBRARY

Patient Care Section

The Patient Care Section conducts the daily operation of the NIDR Dental Clinic and is the focus of clinical oral and dental health concerns at NIH. The Section provides a wide range of diagnostic consultative services to NIH clinical care and research programs. Staff dentists and dental hygienists routinely participate in medical rounds and patient care conferences integrating oral health care concerns to total patient management.

The Section's staff continue their deep commitment to the Dental Staff Fellow program. They are primarily responsible for the clinical training, and introduction to clinical research, of the Dental Staff Fellows. Scheduled rounds, a Fellowship lecture series and a journal review are conducted weekly throughout the year. The lecture series brought in speakers from outside the NIH as well as from laboratories and branches of the various institutes of NIH including the Intramural Research Program, NIDR.

This past year has seen a considerable expansion of Section research programs. This effort continues to be tangibly aided by a close working relationship with the Clinical Investigations Section, the Clinical Studies Unit as well as by collaborations with other programs at NIH. Several major protocol based study areas have been developed and are central to this Section. One addresses management problems of compromised patients undergoing cytotoxic chemotherapy and therapeutic radiation. Because of studies in the NCI, this group forms a large percentage of Section patients. Thus, we are in a unique position to be able to evaluate the efficacy of different regimens of oral health care in a rigorous, controlled fashion and to assess the influence of such regimens on the general health status of these compromised patients. Another general area involves patients with congenital disorders; particular attention being directed towards the characterization and management of dental-craniofacial manifestations. For example, Section staff are involved in efforts to better diagnose patients with ectodermal dysplasia, osteogenesis imperfecta, neurofibromatosis, precocious puberty and nevoid basal cell carcinoma syndrome through roentgencephalometric analyses. Expanded management research is also directed at standardizing fixation methods following orthognathic surgery, at rigorously evaluating endosseous titanium implants in edentulous persons including persons with congenitial dental agenesis such as ectodermal dysplasia, and evaluation of composite resins as posterior dental restorative materials.

Importantly, Section staff have assumed a prominent role in several projects in collaboration with the Clinical Studies Unit. For example, a major focus of Branch AIDS research is directed at understanding the development of oral candidiasis and its relationship to the pathogenesis of AIDS. We have shown that HIV-I positive patients, who are otherwise healthy and non-medicated, have a mean level of whole saliva candida approximately four log orders greater than normal controls ($\sim$1.3x10^4 cfu/ml versus <1.0x10^0 cfu/ml). In all individuals, the predominant yeast recovered was C. albicans. These findings (I) suggest high oral yeasts are an early sequela of HIV-1 infection and (2) are consistent with the notion that oral defense mechanisms are compromised early in HIV-1 infection.

A second major research interest in this section is directed at understanding oral physiological status during normal aging. We now have seen more than IIO normal volunteers from the National Institute on Aging's (NIA) Baltimore Longitudinal Study of Aging (BLSA). Each volunteer is subjected to a detailed evaluation of salivary gland, oral motor and oral sensory performance. During this year we have shown that among healthy, non-medicated persons there is no diminution in submandibular/sublingual fluid secretion across the life span. We have also confirmed the constancy of parotid saliva production in different age groups. The results, coupled with studies from other laboratories which demonstrate a 20-35% loss of acinar tissue in older persons, suggest that salivary glands possess a secretory reserve capacity permitting normal fluid transport despite the loss of substantial parenchymal tissue. This suggestion also has important management implications for patients with salivary hypofunction. We have continued our interest in oral motor function during aging. A cross-sectional study of healthy, non-medicated men and women utilized an NIA cohort at the Clinical Center to evaluate the oral-pharyngeal phases of swallowing by ultrasound. These data are now being analyzed. Other studies of oral motor performance, in BLSA subjects, focused on the ability of subjects to rapidly repeat certain speech sounds, /p/, /t/, /k/, /ptk/, i.e. diadochokinetic rates. We detected no alterations in the ability of aged, non-medicated persons to perform this task. In order to more precisely study (visualize and quantify) lip and jaw movements, during the past year we have developed a computer-linked, three dimensional, video imaging system in cooperation with the Clinical Center's Biomechanics Lab. This should be useful in many clinical areas including speech (e.g. to understand compensatory mechanisms in elderly persons), swallowing (in conjunction with our ultrasound studies) and following maxillo-facial surgery (see above; to enhance soft tissue esthetic and functional outcomes).

This year we also have developed an oral sensory evaluation test battery to conveniently evaluate several components of oral sensory performance (taste, tactile [pressure], thermal and textural [viscosity]), and applied it to the BLSA study population. Our initial findings demonstrate consistent differences between age groups in ability to correctly order stimuli of increasing viscosity, modest differences in ordering pressure stimuli, and equivalent performance in judging thermal and taste stimuli. The latter results are consistent with similar findings previously reported by us last year. We also showed that olfactory recognition decreased significantly with age. Thus, our work has shown that no simple description can be applied to oral sensory function during aging.

Section staff are continuing participation in a multi-center study evaluating the effects of the non-steroidal anti-inflammatory drug flurbiprofen on periodontitis in adult subjects. The study is sponsored by the Upjohn Company and is being conducted at four other research centers besides NIDR; Emory University, Harvard University, University of Michigan and University of Texas-San Antonio. Strict clinical and radiological diagnostic parameters are used to determine if flurbiprofen can restrict the progress of advanced periodontal lesions. Preliminary work has also been completed on a study to evaluate an aqueous solution of tranexamic acid as an oral rinse to prevent post-operative bleeding in patients on warfarin anticoagulants that require oral surgery. In addition, a study to evaluate the effects on chemotherapy-induced stomatitis of a twice daily rinsing with 0.12% clorhexidine gluconate is about to be initiated.

The Patient Care Section has also continued its academic affiliations with the Baltimore College of Dental Surgery of the University of Maryland, Georgetown University School of Dentistry, and Montgomery College. These arrangements provide graduate dental students, senior dental hygiene, dental assistant, and dental laboratory students an opportunity to experience alternative practice settings beyond those offered in the school core curriculum, as well as provide our staff with academic clinical dental teaching responsibilites. The Section also participates in the NIH Clinical Electives Program, and this past year two senior dental students (University of Puerto Rico and University of Colorado) served in an eight week program which emphasized providing dental care to medically compromised patients in a hospital environment and an introduction to clincal research.

Clinical Investigations Section

The general theme of this section's investigative efforts is understanding the regulation of membrane-associated, epithelial cell secretory events. The model tissues studied are mammalian salivary glands and focus of study includes (1) understanding the mechanisms involved in controlling trans-acinar cell transport (serosa to mucosa) of fluid and electrolytes; (2) understanding mechanistic steps in gene expression, protein processing, routing and secretion; and (3) understanding the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders. The organizational framework established last year within this Section, ie. the creation of a Membrane Transport Unit and a Clinical Studies Unit, has proven to be both administratively and scientifically valuable.

NIH LIBRARY

Considerable effort is directed at understanding biochemical steps involved in the formation of saliva. It is well accepted that saliva has a critical role in the defense, and functional maintenance, of all oral tissues. Saliva contains water and electrolytes, derived from serum, and specific exocrine proteins synthesized by glandular epithelial cells. Salivary glands are useful models of secretory processes and studies with these glands have proved important to our understanding of basic concepts of secretion and to appreciating pathogenesis in conditions such as cystic fibrosis.

As noted above, one main thrust of study is directed toward understanding secretory events related to water and electrolyte movement. These responses are primarily under α_1-adrenergic and muscarinic-cholinergic control. Because these autonomic receptors elicit their effects in great part due to changes in membrane ion permeability, we have directed considerable effort at clarifying ion fluxes associated with early events in secretion.

Most studies utilize a dispersed acinar cell preparation, and both basolateral and endoplasmic reticulum membrane vesicle preparations, from rat and rabbit parotid glands. Studies have been directed at understanding transport mechanisms for Cl^-, Na^+, K^+ and Ca^{2+}. The accepted model used to describe salivary fluid secretion requires that four plasma membrane ion transport systems act in concert to move Cl^-, and ultimately fluid, from the interstitial space to the luminal space in response to secretory stimuli. These systems are (1) an electroneutral, loop diuretic-sensitive Na/K/Cl cotransporter located in basolateral membranes of the secretory cell, (2) a basolateral Ca^{2+}-activated K^+ channel, (3) an apical Ca^{2+} activated Cl^- channel, and (4) the Na/K ATPase. According to this model the electrochemical gradient for Na^+ generated by the Na/K ATPase allows Cl^- to be driven into the secretory cell against its electrochemical gradient via the cotransporter. Stimulation of the gland results in the elevation of cytosolic Ca^{2+}, opening the K^+ channel (thus increasing extracellular [K] and so facilitating cotransporter function) and opening the Cl^- channel. The latter results in an accumulation of Cl^- in the lumen. Na^+ is thought to follow by leaking through the intercellular tight junctions. The resulting osmotic gradient for NaCl causes a net transepithelial movement of water from interstitium to the lumen.

Previously, we have demonstrated (1) the presence of a Na/K/Cl cotransporter in parotid basolateral membranes and (2) that parotid cells lose Cl^- in response to α_1-adrenergic and muscarinic-cholinergic stimulation. We have continued to make substantial progress. For example, during the past year we showed that the loop diuretic binding site on the Na/K/Cl cotransporter has an essential sulfhydryl group. Thus, treatment of basolateral membrane vesicles with N-ethylmaleimide dramatically reduces both [^{3}H] bumetanide binding and Na/K/Cl cotransport activity. The presence of bumetanide prevents the sulfhydryl inactivation ($K_{0.5} \sim 3\mu M$). We have also achieved considerable success in solubilizing the cotransporter. When basolateral membranes are treated with 0.07% Triton X-100 and centrifuged at high speed, Na/K/Cl-dependent [^{3}H] bumetanide binding is recovered in the supernatant. Although binding activity is labile, it can be stabilized (or recovered) by the addition of Na, K and Cl salts and specific exogenous lipids, most notably phosphatidylinositol. We have begun raising monoclonal antibodies against the solubilized cotransporter for future use in purification, functional and molecular biological studies.

We also have reported the existence of potent Na/H and Cl/HCO_3 exchangers in parotid basolateral membranes and suggested that they may play a role in fluid secretion. We have continued to examine this hypothesis. In one series of studies intracellular pH (pHi) was monitored in parotid acinar cells with a fluoresecent probe, BCECF. Carbachol stimulation of cells produces a gradual rise in pHi (∿0.1 unit by 10 min) by activating the Na/H exchanger. If amiloride was present or if Na^+ was removed, carbachol resulted in a dramatic drop in pHi (0.4 units with a T1/2 = 0.5 min). This was blocked by the muscarinic antagonist atropine and reduced by removal of HCO_3 and by the carbonic anhydrase inhibitor methazolamide. In Cl^- free media this acidification was more pronounced. Together these data suggest that acinar cell acidification stimulated by carbachol is due to a rapid loss of intracellular HCO_3^-. This effect was not inhibited by SITS (a Cl/HCO_3 exchange inhibitor) and could not be sustained in Ca^{2+} free media. Thus it appears HCO_3^- loss is via the apical anion (Cl^-) channel. Under normal physiological conditions the intracellular acidification resulting from HCO_3^- secretion is buffered by the Na/H exchanger.

Our work also shows that the Na/H exchanger has an important role in salivary secretion. Basolateral membrane vesicles prepared from acinar cells treated with carbachol show 2-3 fold higher levels of Na/H exchange activity compared to control vesicles. This is dependent on the [carbachol] ($K_{0.5}$ ∿3μM) and fully blocked by 20μM atropine. The effect is mimicked by α_1-adrenergic stimulation and shows a T1/2 ∿5 min (i.e. similar to the carbachol-induced pH rise). Treatment of cells with a Ca^{2+} ionophore results in a comparable activation of the Na/H exchanger while phorbol esters, which activate protein kinase C, are without effect. Inhibitors of protein kinase C do not blunt the carbachol effect while calmodulin antagonists significantly diminish Na/H exchange activity. In aggregate, the results indicate that muscarinic-receptor stimulation increases Na/H exchanger activity via a Ca^{2+} and calmodulin dependent process. This would increase intracellular HCO_3^- levels and thus increase HCO_3^- dependent fluid secretion.

Further studies of Cl/HCO_3^- exchange showed that in HCO_3^- containing medium, bumetanide (100μM) could not fully block Cl^- entry into acinar cells. Addition of SITS (100μM) to the bumetanide-medium resulted in a complete Cl^- entry block. In HCO_3^- free medium, SITS was without effect and bumetanide fully inhibited Cl^- entry. This suggests that a portion of the Cl^- entry into acinar cells after carbachol stimulation (∿20%) occurs via Cl/HCO_3 exchange.

We have also begun to carefully examine the relationship between mobilization of intracellular Ca^{2+} and the opening of the basolateral K^+ channel after muscarinic stimulation. We have employed digital video imaging microscopy (with the dye fura-2) and simultaneous recording of acinar cell intracellular potential with voltage sensitive microelectrodes. We observed that a general rise in acinar cell [Ca^{2+}] does not precede activation of the K^+ conductance. Increased K^+ conductance occurs even in Ca^{2+} depleted media but was eliminated if cells were first loaded with the Ca^{2+} chelator BAPTA and then stimulated in Ca^{2+} depleted media. This is consistent with an hypothesis that muscarinic stimulation results in a rapid, localized increase in [Ca^{2+}] near the basolateral membrane. This localized increase cannot be detected by fura-2 but is sufficient to open the Ca^{2+} sensitive K^+ channels. Thus, our work suggests that the target Ca^{2+} store in these cells must be adjacent to the basolateral membrane.

NIH LIBRARY

It has long been known that Ca^{2+} plays a key rôle in stimulus-secretion coupling and, as noted above, the mobilization of Ca^{2+} is a central event in the fluid secretory process. Accordingly, we continue to make a major effort to understand the mechanisms by which cytosolic Ca^{2+} is regulated in salivary epithelial cells.

During the past year we have characterized, in great detail, the mobilization of intracellular Ca^{2+} by β-adrenergic receptor stimulation. Activation of parotid cells by a β-adrenergic agonist, isoproterenol, induces a transient, 1.6 fold increase in cytosolic Ca^{2+} within 9 seconds, returning to resting levels by 60 sec. This effect is dependent on the [isoproterenol] with an EC_{50}∿10μM. The response is not altered by removal of extracellular Ca^{2+}, indicating that an intracellular Ca^{2+} pool is mobilized, and it is completely blocked by a β_2-, but not β_1-, adrenergic antagonist. The pool of Ca^{2+} mobilized is a portion of that mobilized following muscarinic-cholinergic stimulation since the two responses are not additive. In addition, if cells in Ca^{2+}-free medium are first treated with isoproterenol subsequent exposure to carbachol results in a diminished muscarinic response. 8-Bromo cAMP elicts a Ca^{2+} response by parotid cells which is similar to that of isoproterenol.

We also examined whether β-adrenergic receptor stimulation was capable of activating phospholipase C with the subsequent generation of inositol trisphosphate (IP_3). Isoproterenol treatment of parotid cells resulted in ∿50% increase in IP_3 formation above control levels within 30 sec. This response (1) was dependent on the [isoproterenol] with an EC_{50}∿10μM, (2) was blocked by propranolol and (3) was mimicked by analogs of cAMP. Carbachol-induced IP_3 formation was ∿3.5 fold greater than control levels at 30 sec. These data demonstrate that β-adrenergic stimulation of rat parotid acinar cells results in IP_3 formation and the mobilization of a carbachol-sensitive intracellular Ca^{2+} pool by a mechanism involving cAMP. These studies provide a clear demonstration of an interaction (cross-talk) between the cAMP and phosphoinositide messenger systems in these cells.

Additional studies have probed specific signalling mechanisms of autonomic receptor mobilization of parotid intracellular Ca^{2+}. We have observed that aluminum fluoride induces a slow rise in cytosolic free Ca^{2+}, apparently via direct activation of a transducing G protein. Atropine, the muscarinic-antagonist, is capable of blunting this effect. Normally, atropine inhibits carbachol (10μM)-induced Ca^{2+} mobilization at an IC_{50} ∿0.01μM with a maximal effect by 0.1μM antagonist. The maximal effect on the aluminium fluoride response requires 20μM atropine. Surprisingly 20μM atropine also blocked the Ca^{2+} mobilizing and IP_3 generating effects of isoproterenol and 8-bromo-cAMP, but was without effect on any responses elicited by α_1-adrenergic receptor stimulation by epinephrine. This suggests that 20μM atropine is acting in a somewhat specific manner. Considerably more study is required but our results thus far imply that antagonist binding to the muscarinic receptor may induce negative, and novel, post-receptor effects on the signal transduction pathway.

We have also confirmed the hypothesis that α_1-adrenergic stimuli utilize the same intracellular Ca^{2+} target pool as muscarinic and β-adrenergic agonists. The α_1-adrenergic Ca^{2+} mobilizing response is generally similar to that elicited by muscarinic agonists. Peak cytosolic [Ca^{2+}] are ∿3-4 fold resting values, are reached within 5 sec of agonist addition and return to basal levels slowly (∿10 min). When the intracellular Ca^{2+} pool is partially depleted by a sub-maximal [carbachol], 0.2μM, in a low Ca^{2+} medium, maximal epinephrine (10μM)-induced changes in cytosolic Ca^{2+} were substantially decreased.

We have continued to utilize a naturally occuring model of perturbed α_1-adrenergic signalling, the aged rat parotid gland, to understand some differences in transducing mechanisms between α_1-adrenergic and muscarinic receptors. We have previously reported that there is a marked reduction in α_1-adrenergic, but not muscarinic, Ca^{2+} mobilizing responsiveness with senescence. This difference is not due to a reduction in ability to generate IP_3 after α_1-adrenergic stimulation, nor is it related to any alteration in the ability of the presumed intracellular target pool (endoplasmic reticulum) to accumulate Ca^{2+} via the ATP-dependent Ca^{2+} transporter. In aggregate, our studies suggest that the α_1-adrenergic receptor likely mobilizes Ca^{2+} by involving an additional second messenger(s) besides IP_3, which is not shared with the muscarinic receptor.

ATP-Dependent Ca^{2+} transporters (Ca^{2+} pumps) play a major role in the maintenance of cellular Ca^{2+} homeostasis. There are two Ca^{2+} pumps in parotid acinar cells, one each in the basolateral plasma membrane and in the endoplasmic reticulum membrane. The former has a major role in extruding Ca^{2+} from the cell against a large concentration gradient (extracellular free Ca^{2+} is ∿10^4 fold greater than intracellular free Ca^{2+}), while the latter is known to be responsible for maintenance of Ca^{2+} levels in the signal target pool. Over the last several years we have focused considerable effort on characterizing these pumps. During this reporting period we have examined the regulation of the basolateral membrane pump by calmodulin (CAM). At ∿100nM Ca^{2+}, CAM-stimulates ATP-dependent transport activity by ∿60%. CAM increases both the affinity of the transporter for Ca^{2+} (reduced K_m ∿40%) and the maximum transport capacity (V_{max}, increased ∿20%). Treatment of native membranes with EGTA to remove CAM, reduces Ca^{2+} affinity. This is restored when CAM is readded to the membranes. In addition, we showed that several CAM antagonists inhibit basolateral membrane ATP-dependent Ca^{2+} transport. These results demonstrate that the rat parotid basolateral membrane Ca^{2+} pump can be significantly regulated by CAM at typical cytosolic [Ca^{2+}].

We have also assessed the ability of these pumps to function at physiological resting [Ca^{2+}], and at [Ca^{2+}] which result from stimulation. We performed detailed kinetic studies over a broad range of [Ca^{2+}]. Cytosolic resting [Ca^{2+}] in parotid acinar cells is ∿100-200nM, and peaks, following α_1-adrenergic or muscarinic-cholinergic stimulation, at ∿800nM. Using isolated vesicle preparations, we showed that ATP-dependent Ca^{2+} transport in the basolateral membrane (BLM) exhibits a K_m∿75-80nM Ca^{2+} while V_{max} is achieved at [Ca^{2+}]>800nM. The Ca^{2+} pump of the endoplasmic reticulum (ER) exhibits a Km ∿20-40nM Ca^{2+} and is fully activated at ∿100nM. The ER pump is thus completely functional even under resting conditions and is unlikely to be affected by the elevated cytosolic [Ca^{2+}] which accompanies secretory stimuli. Conversely the BLM pump is kinetically regulated within the physiological range of cytosolic [Ca^{2+}] to which parotid cells are exposed. Together with our finding that CAM increases the affinity of this pump for

NIH LIBRARY

Ca^{2+}, these kinetic results suggest that the BLM transporter should be rendered more sensitive to lower [Ca^{2+}] and thus able to function more efficiently under sustained stimulation conditions.

A noticeable major area of progress this year has been the continued development and utilization of cell lines derived from salivary epithelia for studying receptor-coupled physiological events. In general, there exist relatively few permanent cell lines derived from epithelial (vs. mesenchymal) tissues. Primary cultures of differentiated epithelial cells also have been difficult to develop and maintain. We have extended our earlier attempts to establish parotid acinar cells in primary culture in two ways. First, we have succeeded in growing epithelial cultures from parotid glands of several species, including rat, rabbit, cow and minipig, in low-serum or serum free conditions. We are now engaged in studies to characterize these cell types. Secondly, we have been successful in applying and modifying techniques originally devised by Dr. C. Oliver (LI,NIDR) to prepare long-term cultures of parotid acinar cells. These cells remain highly granulated and well-differentiated up to 86 days in culture (the longest time studied). By Western blot analysis, the cells continue to secrete both amylase and proline-rich proteins and thus appear, to be a useful model for studying the regulation of specific parotid gene expression.

Most importantly, we now have, in routine use, several established salivary cell lines which have shown considerable promise for characterizing neurotransmitter responses. Established cell lines offer several advantages over acute in vitro preparations including (1) the ability to carry out long term studies, (2) the convenience and reproducibility which comes with cloned cell lines, and (3) the general applicability to cell cultures of somatic cell genetic and molecular biological techniques. The following four cell lines have been studied: A253, isolated from an adenocarcinoma of a human submandibular gland; A5, a cloned rat submandibular ductal cell line derived from RSMT cells which we have previously reported; HSG-PA, a cell line derived from the intercalated duct region of a human submandibular gland; and HSG-MY, derived from HSG-PA following chronic exposure to sodium butyrate and purported to be a myoepithelial cell. Most studies during this year, have employed HSG-PA and A5 cells as well as mutant cell lines derived from these parental lines.

Considerable effort has focused on HSG-PA cells because they are structurally well-characterized and, by immunologic and electron microscopic criteria, appear to be derived from intercalated duct cells. This segment of a salivary gland is of considerable interest because it is (i) purportedly a progenitor cell-type giving rise to acinar cells and (ii) essentially inaccessible for study by conventional acute preparation techniques. We have shown that HSG-PA cells possess high-affinity muscarinic-cholinergic receptors by [^{3}H]-QNB binding (K_d=0.17nM; B_{max}=37 fmol/mg protein) but not α- or β-adrenergic receptors. The muscarinic agonist carbachol elicits a concentration dependent increase in IP_3 formation (>2 fold in 30 sec at 100μM) demonstrating that the muscarinic receptors are functionally coupled. Carbachol also results in ∿5 fold increase in cytosolic Ca^{2+}. This response includes a component representing release of intracellular Ca^{2+} and a component representing extracellular Ca^{2+} entry. Ca^{2+} release is quite rapid, being ∿6 fold greater than the rate of extracellular Ca^{2+} entry and 2-3 fold more in magnitude. Ca^{2+} entry is blocked by both La^{3+} and atropine but unaffected by several voltage-dependent Ca^{2+} channel antagonists and by membrane depolarization. We also examined the effects of phorbol myristate acetate (PMA) on carbachol-induced Ca^{2+} mobilization in these cells. PMA (0.1μM) blocked intracellular Ca^{2+} release and extracellular Ca^{2+} entry. Another phorbol ester, 4α-phorbol 12,13 didecanoate, which does not activate protein kinase C, was without effect on Ca^{2+} mobilization. At 1nM, PMA blocked Ca^{2+} release but not Ca^{2+} entry. The latter result suggests that after muscarinic receptor stimulation Ca^{2+} entry does not occur via an intracellular store. In addition, protein kinase C appears to play a role in a feedback control mechanism of Ca^{2+} mobilization at different levels.

We have also shown that muscarinic stimuli cause changes in the transport of ^{86}Rb, ^{36}Cl and ^{22}Na across HSG-PA cell plasma membranes. Most studies have concentrated on ^{86}Rb transport. Carbachol increases both ^{86}Rb influx and efflux but the steady-state [^{86}Rb] within cells remains unchanged. These effects are dependent on the [carbachol] and fully blocked by atropine. Neither ouabain (an inhibitor of the Na/K ATPase) nor bumetanide (an inhibitor of the Na/K/Cl cotransporter) inhibit the ^{86}Rb fluxes. However, K channel inhibitors, such as $BaCl_2$, quinine and tetraethylammonium chloride, are effective. ^{86}Rb Fluxes are Ca^{2+} dependent. Removal of extracellular Ca^{2+} blunts ^{86}Rb fluxes while a Ca^{2+} ionophore mimics the response. PMA (0.1μM) can partially block carbachol-stimulated ^{86}Rb fluxes, likely by inhibiting Ca^{2+} mobilization.

Studies this year with A5 cells have concentrated on the β-adrenergic receptor system. Binding of the β-adrenergic antagonist [^3H]-DHA is specific and saturable with respect to time and concentration. There is a single class of high affinity β-adrenergic receptors in A5 cells with a K_d=0.62nM and a B_{max}=101 fmol/mg protein. This receptor is of the β_2 subtype and is functionally coupled to adenylate cyclase since isoproterenol results in a concentration dependent, propranolol blockable, increase in intracellular cAMP (maximal 130 fold increase). β-Adrenergic stimulation of A5 cells also results in marked morphological changes, a ∿20% decrease in cell volume and ∿30% decrease in cell Cl^- content. 8-Bromo-cAMP causes identical effects. The putative Cl^- channel blocker, NPA, substantially inhibits these volume and Cl^- responses.

We also have made considerable progress in employing somatic cell genetic techniques with these cells. We have isolated 5 colonies of HSG-PA cells which are resistant to the cytotoxic effects of high [W-7], a CAM inhibitor. Three different morphological phenotypes are apparent and several of these clones show growth rates significantly altered from the parental line. We also have subcloned the A5 cell line twice using a limiting dilution technique and obtained A531 cells. Mutants of this cell line were selected for their ability to grow in medium containing a low [K]. Approximately 30 mutant colonies have been obtained and several are currently being cloned. All mutants morphologically resemble the parental line. Two mutant lines grow at normal rates in low K media but exhibit markedly reduced growth rates in normal media. Initial studies suggest that mutant cells have ∿30% reduction in cellular K content and fail to respond to β-adrenergic stimulation by reducing intracellular and Cl^- cell volume. These results suggest the promise of using somatic cell mutation techniques to study receptor-controlled ion flux mechanisms in these salivary epithelial cells.

NIH LIBRARY

In addition to secreting water and electrolytes, salivary cells secrete many functionally important exocrine proteins into saliva. We have continued our efforts to understand the regulatory factors involved in the expression, processing, routing and release of secretory proteins. These studies have employed the entire range of experimental models available in our laboratory; cell lines, acute acinar cell preparations and intact animals. For example, we have employed surgical and pharmacological (reserpine) sympathectomy to study possible receptor modulation in parotid acinar cells and its relationship to protein exocytosis. Cells from reserpinized rats show reduced responses to β-adrenergic stimulation in vitro compared to controls (∿70%). 8-Bromo-cAMP was unable to surmount this reserpine effect, a result which suggests that reserpine inhibition does not alter receptor: adenylate cyclase coupling. Cells prepared from surgically sympathectomized rats display only a slight decrease in protein secretion compared to controls (∿25%). In membranes from reserpinized rats there was a modest increase in β-adrenoreceptor number (∿35%), while no such increase was seen after surgical treatment. No shift in receptor subtypes (β_1 vs β_2) was observed. Thus, short term surgical sympathectomy of adult rats produced only slight changes in β-adrenergic receptors and regulated secretion. In contrast, reserpine treatment markedly impaired the ability of the parotid glands to respond to stimulation, suggesting that drug action is distal to receptor: cyclase coupling and most likely unrelated to pharmacological denervation.

β-Adrenergic receptor activation also has profound effects on parotid acinar cell gene expression. Last year we reported that following β-adrenergic stimuli, parotid acinar cells showed enhanced expression of several oncogenes encoding nuclear proteins (c-fos, c-myc, p53) and two growth factors (c-abl, c-sis). During this year we have focused our efforts primarily on c-fos expression. We have shown that increased c-fos gene expression is accompanied by an increase in the production of its protein product. Using immunocytochemical methods, we showed that c-fos protein staining was evident over the nuclei of parotid acinar cells treated with isoproterenol, while control cells exhibited no antibody staining. We also examined the hypothesis that c-fos expression is related to proliferative responses by cells. We used two novel experimental approaches to address this hypothesis in rat parotid; (i) specific pharmacological stimuli and (ii) increased chronological age. For (i), parotid acinar cells were incubated with different neurotransmitter agonists. Both isoproterenol (β-adrenergic) and carbachol (muscarinic-cholinergic) markedly, and equivalently, increased c-fos expression over control levels. The equipotent effectiveness of these agents is not consistent with their tissue proliferative effects. Only β-adrenergic agonists markedly increase DNA synthesis, and elicit tissue hyperplasia, in rat parotid gland in vivo. Muscarinic agonists are incapable of this effect. These results show that c-fos gene expression can be dissociated from DNA synthesis and show that the c-fos gene is inducible in rat parotid cells by receptor systems coupled to two distinct transduction mechanisms: adenylate cyclase (β-adrenergic) and phosphoinositide specific phospholipase C (muscarinic). For (ii) we employed parotid acinar cells prepared from 2 and 12 month rats, treated ± isoproterenol. In both cell groups the β-adrenergic agonist elicited comparable levels of c-fos expression. However, in vivo parotid cells from 12 mos animals show a marked reduction in DNA synthesis following β-adrenergic stimuli, compared to the response of cells from 2 mos animals. Thus DNA synthesis can also be dissociated from c-fos expression by this aging paradigm.

A similar series of experiments was carried out using the A5 salivary epithelial cell line. This has the advantage of using the same experimental preparation for both c-fos expression and DNA synthesis studies. Both isoproterenol (10µM) and 8-bromo-cAMP (1mM) markedly increase (∿5.5 fold) c-fos mRNA levels above controls within 30 min and these remain elevated up to 60 min. Thereafter steady-state levels of c-fos mRNA gradually decline to basal levels. No c-fos transcripts were detected in cells exposed to either agent for 4-24 hrs. This appears to be a somewhat specific event since no detectable changes in p53 expression were observed throughout the experimental time course. When [^{3}H]-thymidine incorporation into DNA was followed, A5 cells treated with these agents showed a significant reduction of isotope incorporation up until 2 hours. After 4 hours of treatment, [^{3}H]-thymidine incorporation was at control levels and remained so up to 24 hrs. These findings show that the regulation of c-fos expression in A5 cells by β-adrenergic stimuli is not directly related to proliferative responses.

Earlier studies on the localization and packaging of secretory protein in the rat submandibular gland also have progressed considerably. Using EM immunocytochemistry we have studied the distribution of specific proteins (neonatal protein B_1; neonatal protein C; mucin glycoprotein; glutamic acid and glutamine-rich protein, GRP) to follow the changing cell populations during submandibular gland development. From their first appearance in fetal glands, proteins B_1 and C are localized to separate cells. Following birth, type III cells begin to show the presence of both proteins in their granules. This increases up to day 7 when clear precursors of adult mucous acinar cells can be identified. Secretory granules in the latter cells are labeled with antibodies to proteins B_1, C, mucin and GRP. About day 20, cells no longer label with antibody to protein B_1. Parallel studies show that mRNA for GRP is detectable in these cells by hybridization studies from 3 days after birth and continues to increase up to day 60. These studies confirm our earlier hypothesis that adult mucous acinar cells originate with neonatal type III cells. Using similar approaches we have studied the lineage of intercalated duct cells. These studies suggest that the intercalated ducts are a mosaic of at least two cell types plus perhaps an additional, less differentiated precursor cell.

We have also continued our studies examining the role of salivary duct cells in protein reabsorption. Earlier we showed that following retrograde ductal infusion of exogenous proteins, there is a large uptake of these proteins by striated and intercalated ducts. The internalized proteins are located in large apical vacuoles. We hypothesized that the mechanism by which the duct cells internalize these proteins may include a carbohydrate recognition step. Our results this year support this hypothesis. Uptake by duct cells of mannose-bovine serum albumin (BSA) and fucose-BSA was significantly greater than that of native BSA. The uptake of mannose-BSA was blocked by infusion in the presence of excess mannose or mannan. Thus, it appears that the luminal surface of duct cells possesses a lectin - like receptor with a specificity similar to that of the mannose receptor on human and rat macrophages.

The focus of this Section's human clinical research is understanding the etiology and oral sequelae of conditions which result in salivary dysfunction. Central to this activity is the dry mouth, xerostomia study which now has involved over 550 patients. We have established diagnostic approaches to the evaluation of patients with complaints suggestive of salivary gland dysfunction. Most patients seen have diminished gland secretory capacity. About sixty individuals per year are admitted as inpatients for intensive study under the protocol "Evaluation and treatment of salivary gland dysfunction". An approximately equal number are studied as outpatients. We have continued two treatment protocols for salivary dysfunction which were begun last year. In one protocol, we utilize a steroid (prednisone), and a non-steroidal anti-inflammatory drug (peroxicam), to control the autoimmune damage to salivary glands in primary Sjogren's syndrome. There are approximately 1-2 million Americans with primary Sjogren's syndrome and as yet no specific therapy exists for the condition. Patients with early and late stage disease are studied. Treatment is designed to both arrest progessive gland dysfunction and improve existing function. Early results (∿10 patients) are encouraging. In the other protocol, we employ the parasympathomimetic drug pilocarpine to preserve salivary function during head and neck irradiation. Approximately 50,000 Americans each year are diagnosed with a head and neck cancer. Most are treated, at least in part, with irradiation. The single greatest area of post-treatment complaint in surviving patients is related to oral damage reflective of salivary dysfunction. Thus far six patients have begun this protocol. Owing to the double blind design, no results are available yet. Both of these protocols address significant clinical management problems. In addition, we have almost completed the third trial study of pilocarpine, reported last year. In this study, patients with gland hypofunction, but with evidence of residual gland parenchyma present, receive pilocarpine three times per day over a 6 month period (one-month is a double-blind placebo period). All required patients have been enrolled and only a few months remain until completion of this trial. Thereafter, full analysis of data will begin.

A major strength of our branch is the linkage of our laboratory and clinical components. Because of (1) our location, within the NIH Clinical Center, (2) our mixture of basic scientists and clinicians, and (3) the close physical approximation between our various groups, we are able to enhance each other's research effort and fulfill our mission. As noted earlier, two major areas of clinical research interest involve aging and AIDS. Our accomplishments in aging were described under the Patient Care Section (above), but it should be stressed that this project involves a close working relationship with personnel in the Clinical Investigations Section. This is true also of AIDS studies. Our studies of oral candidais in AIDS patients were described above. These studies are part of an increasing focus of our branch on compromised oral defense mechanisms in AIDS patients. A significant part of these studies involves salivary gland status. We previously reported specific defects in salivary gland function in asymptomatic, non-medicated AIDS patients (ie. early in the disease course). Of note, these changes were predominantly associated with the submandibular/sublingual glands and included significant elevation in salivary lysozyme levels and a marked increase in the frequency with which albumin was detected in the secretion. These studies have been extended this past year. We have shown consistent and significant elevations in several anti-microbial factors in submandibular/sublingual saliva. These include beside lysozyme, lactoferrin and secretory IgA. No changes were seen in another group of important salivary proteins, the proline-rich-proteins, which have no anti-microbial role. Again, patients were selected with extreme care, to eliminate medication effects and underlying infections as variables and to enable us to study early stage, asymptomatic individuals. Our results suggest the possibility that early in the disease process a compensatory protective response may be occurring in the submandibular/sublingual glands.

A major finding by us during this past year was the identification of a component in saliva which is capable of inhibiting HIV-I infection of stimulated human peripheral blood lymphocytes. Individual gland secretions, as well as whole saliva, were obtained from 3 healthy, non-medicated control men. They did not belong to any known risk group for AIDS. HIV-I, after preincubation with whole saliva for 1hr, was unable to infect lymphocytes. Most of this inhibitory activity was found to reside in submandibular/sublingual saliva, while parotid samples were inconsistent in effect on HIV-I infectivity. These studies provide objective support for the considerable but indirect evidence that saliva is not a route for transmission of HIV-I.

In a related project, we have begun studying another important component of oral defense, the mucosal barrier. We initially evaluated the oral mucosal status of control individuals and patients with salivary hypofunction by using a semi-quantitative clinical rating scale and clinical description. We observed that patients had a generally intact oral mucosa even in the presence of significant major gland hypofunction. The patient group did have a significant increase in mild oral mucosal alterations compared to controls. However, patients showed no differences in frequency of severe mucosal changes or infections. This surprising finding lead us to hypothesize that additional factors, not limited to major gland secretions, may play a role in maintenance of the oral mucosal barrier. This hypothesis was encouraged by the recent finding of a group in NICHD which showed that frog skin contained a group of endogenous anti-microbial peptides, termed magainins. In an excellent example of cooperation within the CIPCB, the suggestion that magainin-like compounds may be found in the oral cavity was tested. Considerable effort was directed at determining if magainin-like activity could be extracted from buccal mucosa. We chose to test hamster mucosa and, although we have developed extremely sensitive plate assays for measuring anti-microbial activity, none was found. We have, however, demonstrated the presence of magainin-like immunoreactivity in frozen sections of human minor salivary glands as well as detected magainin cross-reacting material, of the appropriate molecular size, on Western blots of labial gland saliva samples. We thus have established in our branch a comprehensive program to evaluate the major components of oral defense (saliva, commensal flora, mucosal barrier) and study their perturbation during various pathologic conditions.

Success in the unique mission of the Clinical Investigations and Patient Care Branch is made possible by the blending of the academic, clinical problem-oriented Patient Care Section together with the strongly basic science-oriented, yet clinical problem appreciating, Clinical Investigations Section. We continue to make substantial progress since the reorganization of the Branch in 1982. We have the opportunity to make many contributions to clinical dentistry, to oral science as well as to fundamental biology. Our Branch recognizes this opportunity and works creatively and with considerable enthusiasm and effort toward meeting this goal. We anticipate continued forward movement in our efforts to address questions of importance to the understanding and management of oral diseases, thus contributing to the future development and direction of the dental profession.

CLINICAL INVESTIGATION AND
PATIENT CARE BRANCH
PUBLICATIONS 1987-1988

Ambudkar IS, Abdallah El-Sayed, Shamoo AE. Lysophospholipid-mediated alterations in the calcium transport systems of skeletal and cardiac muscle sarcoplasmic reticulum, Mol Cell Biochem 1988;79:81-9.

Ambudkar IS, Baum BJ. ATP-dependent Ca^{2+} transport in rat parotid basolateral membrane vesicles is modulated by membrane potential, J Membrane Biol 1988;102:59-69.

Ambudkar IS, Baum BJ. Modulation of ATP-dependent Ca^{2+} transport in rat parotid basolateral membrane vesicles by $K^{+}+Cl^{-}$ flux, Biochim Biophys Acta (in press).

Ambudkar I.S, Kuyatt BL, Roth GS, Baum BJ. Modification of ATP-dependent basolateral membranes during aging, Mech Ageing and Develop 1988;43:45-60.

Ambudkar IS, Melvin JE, Baum BJ. α_1-Adrenergic regulation of Cl^{-} and Ca++ movements in rat parotid acinar cells, Pflugers Archiv (in press).

Ambudkar IS, Smith MW, Phelps PS, Regec AL, Trump BF. Extracellular Ca^{2+}-dependent elevation in cytosolic Ca^{2+} potentiates $HgCl_2$-induced renal proximal tubular cell damage, Toxicology and Industrial Health 1988;4:107-23.

Ball WD, Hand AR, Johnson AO. Secretory proteins as markers for cellular phenotypes in rat salivary glands, Dev Biol 1988;125:265-79.

Ball WD, Hand AR, Moreira JE, Johnson AO. A secretory protein restricted to type I cells of neonatal rat submandibular glands, Dev Biol (in press).

Banerjee DK, Kousvelari EE, Baum BJ. cAMP-Mediated Protein Phosphorylation of Microsomal Membranes Modulates Man-P-Dol Synthase, Proc Natl Acad Sci 1987;84:6389-93.

Baum BJ. Saliva secretion and composition. In: Ferguson DB, ed. Frontiers in Oral Physiology vol. 6 - The Aging Mouth. Basel: Karger 1987;126-134.

Baum BJ. Regulation of salivary secretion. In: Sreebny LM, ed. The Salivary System: salivary glands and saliva. Boca Raton: CRC Press, 1987;123-134.

Baum BJ. The Oral Cavity. In: Rowe J, Besdine R, eds. Geriatric Medicine. 1988;157-166.

Baum BJ, Ambudkar IS. Regulation of calcium handling by rat parotid acinar cells, Mol Cell Biochem (in press).

Baum BJ, Burstone CJ, Dubner R, Goldhaber P, Levine MJ, Loesche WJ, Terranova V. Advances in diagnosis and detection of oral disease, Adv Dent Res (in press).

Baum BJ, Fox PC. Chemistry of Saliva. In: Talal N, Moutsopoulus HM, Kassan S, eds. Sjogren's syndrome: clinical and immunological aspects. Berlin: Springer-Verlag, 1987; 34-40.

Baum BJ, Ambudkar IS, Helman J, Melvin JE, Turner RJ. Dispersed salivary gland acinar cell preparations for use in studies of neuroreceptor coupled secretory events, Methods Enzymol (in press).

Baum BJ, Ambudkar IS, Horn VJ. ATP-dependent Ca^{2+}-Transport in rat parotid microsomal membranes requires change compensation, Biochem J (in press).

Baum BJ, Roberts MW, Brahim JS, Fox PC. The National Institute of Dental Research Clinical Dental Staff Fellowship, J Dent Educ (in press).

Brahim JS, Katz RW, Roberts MW. Non-Hodgkins lymphoma of the hard palate, mucosa and buccal gingiva associated with AIDS, J Oral Max Surg 1988;49:328-30.

Coleman R, Hand AR. Endocytosis of native and cationized ferritin by intralobular duct cells of the rat parotid gland, Cell Tissue Res 1987;249:577-86.

Delisle AL, Donkersloot JA, Kolenbrander PE, Tylenda CA. Use of lytic bacteriophage for Actinomyces viscosus T14V as a probe for cell surface components mediating intergeneric coaggregation, Infect Immun 1988;56:54-9.

Field RB, Hand AR. Secretion of lingual lipase and amylase from rat lingual serous gland, Am J Phsiol 1987;253:G217-25.

Fox PC, Busch KA, Baum BJ. Subjective reports of xerostomia and objective measures of salivary gland performance, J Amer Dent Assoc 1987;115:581-4.

Fox PC, Heft MW, Herrera M, Bowers MR, Mandel ID, Baum BJ. Secretion of anti-microbial proteins from the parotid glands of different aged healthy persons, J Gerontology 1987;42:466-9.

Fox PC, Sarras AK, Bowers MR, Drosos AA, Moutsopoulos HM. Oral and sialochemical findings in patients with autoimmune rheumatic disease, Clin Exp Rheum 1987;5:123-6.

Fox PC, Wolff A, Yeh C-K, Atkinson JC, Baum BJ. Saliva inhibits HIV-1 infectivity, J Amer Dent Assoc 1988;116:635-7.

Galili D, Marmary Y. Juvenile recurrent parotitis: Clinicoradiologic follow-up study and the beneficial effect of sialography. Oral Surg Oral Med Oral Path 1987;61:550-6.

Hand AR. Functional ultrastructure of the salivary glands. In: Sreebny LM, ed. The salivary system: salivary glands and saliva. Boca Raton: CRC Press, 1987;43-67.

Hand AR. The secretory process of salivary glands and pancreas. In: Riva A, Motta P, eds. Ultrastructure of the extraparietal glands of the alimentary canal. The Hague: Martinus Nijhoff (in press).

Hand AR, Ball WD. Ultrastuctural immunocytochemical localization of secretory proteins in autophagic vacuoles of parotid acinar cells of starved rats, J Oral Path (in press).

He X, Ship J, Wu X, Brown AM, Wellner RB. β-Adrenergic regulated chloride content in an established rat submandibular cell line in culture, J Cell Physiol (in press).

He X, Wu X, Baum BJ. Protein kinase C differentially inhibits muscarinic receptor operated Ca^{2+} release and entry in human salivary cells, Biochem Biophys Res Commun 1988;152:1062-69.

He X, Wu X, Brown AM, Wellner RB, Baum BJ. Characteristics and metabolism of α_1-adrenergic receptors in an established salivary cell line, RSMT-A5, Gen Pharmacol (in press).

Helman J, Ambudkar IS, Baum BJ. Adrenoreceptor mobilization of calcium in rat submandibular cells, Eur J Pharmacol 1987;143:65-72.

Herrmann HJ, Roberts MW. Preventive dental care: the role of the pediatrican, Pediatrics 1987;80:107-10.

Horn VJ, Baum BJ, Ambudkar IS β-Adrenergic receptor stimulation induces inositol trisphosphate production and Ca^{2+} mobilization in rat parotid acinar cells, J Biol Chem (in press).

Ishikawa Y, Gee MV, Ambudkar IS, Bodner L, Baum BJ, Roth GS. Age-related impairment in rat parotid cell α_1-adrenergic action at the level of inositol trisphosphate responsiveness, Biochim Biophys Acta 1988;968:203-10.

Koss E, Weiffenbach JM, Haxby JV, Friedlander RP. Olfactory detection and identification are dissociated in Alzheimer's Disease, Neurology (in press).

Kousvelari EE, Banerjee DK, Baum BJ. β-Adrenoreceptor regulation of N-linked protein glycosylation in young adult and aged rat parotid gland cells in vitro, Mech Ageing Develop 1988;42:173-81.

Kousvelari EE, Banerjee DK, Grant SR, Baum BJ. β-Adrenoreceptor activation accelerates oligosaccharide processing in an exocrine secretory glycoprotein of rat parotid cells, Archs Oral Biol 1988;33:115-20.

Kousvelari EE, Ciardi JE, Bowers MR. Altered bacterial aggregation and adherence associated with changes in rat parotid-gland saliva proteins induced in vivo by β-adrenergic stimulation, Archs Oral Biol 1988;33:341-6.

Kousvelari EE, Louis JM, Huang L-H, Curran T. Regulation of proto-oncogenes in rat parotid acinar cells in vitro after stimulation of β-adrenergic receptors, Expt Cell Res (in press).

Lotti LV, Hand AR. Endocytosis of parotid salivary proteins by striated duct cells in streptozotocin-diabetic rats, Anat Rec (in press).

Manganel M, Turner RJ. Coupled Na/H exchange in rat parotid basolateral membrane vesicles, J Membrane Biol (in press).

Marmary Y, Fox PC, Baum BJ. Fluid secretion rates from mouse and rat parotid glands are markedly different following pilocarpine stimulation, Comp Biochem Physiol 1987;88A:307-10.

Mednieks MI, Hand AR. Cyclic AMP-dependent protein kinase compartmentalization in stimulated exocrine cells, Ann NY Acad Sci 1987;494:135-7.

Mednieks MI, Hand AR. Salivary gland ultrastructure and cyclic AMP-dependent reactions in Spacelab 3 rats, Am J Physiol 1987;252:R233-9.

Mednieks MI, Jungmann RA, Hand AR. Ultrastructural immunocytochemical localization of cyclic AMP-dependent protein kinase regulatory subunits in rat parotid acinar cells, Eur J Cell Biol 1987;44:308-17.

Melvin JE, He X, Baum BJ. Sympathetic denervation fails to produce β-adrenergic supersensitivity in adult rat parotid gland, J Pharmacol Exp Thera (in press).

Moran A, Davis LJ, Turner RJ. High affinity phlorizin binding to the LLC-PK1 cells exhibits a sodium:phlorizin stoichiometry of 2:1, J Biol Chem 1988;263:187-92.

Roberts MW, Brahim JS, Rinne NF. Oral manifestations in AIDS: a study of 84 patients, J Amer Dent Assoc 1988;116:863-6.

Roberts MW, Shou Hua, Li. Oral findings in anorexia nervosa and bulimia nervosa: a study of 47 cases, J Amer Dent Assoc 1987;115:407-10.

Scott J, Baum BJ. Oral effects of Ageing. In: Jones H, Mason D, eds. Oral manifestations of systemic disease (in press).

Shiraki M, Ishikawa Y, Baum BJ, Roth GS. Effects of aging on parathyroid hormone stimulated ionic fluxes in rat parotid cell aggregates, Mech Ageing Develop 1987;40:215-22.

Silbermann M, Reddi AH, Hand AR, Leapman RD, von der Mark, K, Franzen A. Further characterization of the extracellular matrix in the mandibular condyle in neonatal mice, J Anat 1987;151:169-88.

Smith MW, Ambudkar IS, Phelps PC, Regec AL, Trump BF. $MgCl_2$-induced changes in cytosolic Ca^{2+} of cultured rabbit renal tubular cells, Biochim Biophys Acta 1987;931:130-42.

Sonies BC, Parent LJ, Morrish K, Baum BJ. Durational aspects of the oral phase of swallow in normal aging adults, Dysphagia (in press).

Sonies BC, Baum BJ. Evaluation of swallowing pathophysiology, Otolaryngol Clin North America (in press).

NIH LIBRARY

Turner RJ. Stoichiometry of coupled transport systems in vesicles, Meth Enzymol (in press).

Turner RJ, George JN. Cl/HCO_3 exchange is present with Na/K/Cl cotransport in parotid acinar basolateral membranes, Am J Physiol 1988;254:C391-96.

Turner RJ, George JN. Ionic dependence of bumetanide binding to the rabbit parotid Na/K/Cl cotransporter, J Membrane Biol. 1988;102:71-77.

Tylenda CA, Ship JA, Fox PC, Baum BJ. Evaluation of submandibular salivary flow rate in different age groups, J Dent Res (in press).

Van Story-Lewis PE, Roberts MW, Klippel JH. Oral effects of steroid therapy in a patient with system lupus erythematosus: a case report, J Amer Dental Assoc 1987;115:49-51.

Weiffenbach JM. Taste perception mechanisms. In: Fergusion DB, ed. Frontiers in Oral Physiology: The Aging Mouth. Basel: S Karger, 1987;6:151-67.

Weiffenbach JM. Assessment of chemosensory functioning: Subjective and objective procedures. In: Murphy C, Cain W, eds. Nutrition, the chemical senses and aging. Ann NY Acad Sci (in press).

Weiffenbach JM. Taste changes with aging, Int J Technol Aging (in press).

Weiffenbach JM, Fox PC, Baum BJ. Taste and salivary gland dysfunction. In: Roper S, Atema J, eds. Olfaction and Taste 1X. Ann NY Acad Sci, 1987;510:696-98.

Weiss EI, Kolenbrander PE, London J, Hand AR, Andersen RN. Fimbria-associated proteins of Bacteroides loesheii PK1295 mediate intergeneric coaggregations, J Bacteriol 1987;169:4215-22.

Weiss EI, London J, Kolenbrander PE, Hand AR, Siraganian R. Localization and enumeration of fimbria-associated adhesins of Bacteroides loescheii, J Bacteriol 1988;170:1123-28.

Wellner RB, Ghosh PC, Roechlein B, Wu HC. Perturbation of N-linked oligosaccharide results in an altered incorporation of $[^3H]$-palmitate into specific proteins in chinese hamster ovary cells, J Biol Chem 1987;262:13204-11.

Wellner RB, He X, Marmary Y, Baum BJ. Functional β-adrenergic receptors in a human mammary cell line (HBL-100), Biochem Pharmacol (in press).

Wolff A, Marmary Y, Fox PC. Parotid gland function is unimpaired following acute methotrexate administration in rats. Arch Oral Biol (in press).

Wright WE. Management of oral sequela. J Dent Res 1987;66:699-702.

Wright WE. Periodontium destruction associated with oncology therapy-five case reports, J Periodontology 1987;58:559-63.

Yeh CK, Fox PC, Fox CH, Travis WD, Lane HC, Baum BJ Kaposi's sarcoma of the parotid gland in acquired immunodeficiency syndrome (AIDS)-A case report, Oral Surg (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00028-21 CI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructure and Cytochemistry of Secretory Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hand, Arthur R.	Dental Director	CIPC NIDR
Moreira, Jorge E.	Visiting Associate	CIPC NIDR
Lotti, Lavina V.	Visiting Fellow	CIPC NIDR
Vagman, Ithamar	Guest Researcher	CIPC NIDR
Wolff, Andy	Visiting Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*

See next page

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.93	2.48	1.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic mechanisms of the secretory process are studied in rat and human salivary gland cells. Techniques utilized include light and electron microscopy, enzyme-and immunocytochemistry, radioautography, and biochemistry. Major areas of investigations are: (1) localization of secretory and cellular proteins in developing and adult salivary glands using fluorescent and colloidal gold immunolabeling procedures; (2) structure and permeability properties of junctional complexes in rat salivary glands; and (3) the role of salivary duct cells in protein reabsorption.

PHS 6040 (Rev. 1/84)

GPO 914-918

Cooperating Units

Tabak, L.A., Dept. of Dent. Res., Univ. of Rochester
Malamud, D., Dept. Biochemistry, Univ. of Pennsylvania
Ball, W.D., Dept. of Anatomy, Howard University
Mednieks, M.I., and Jungmann, R.A., Cancer Res. Ctr. Northwestern Univ.
Dowd, F.J., Dept. of Pharmacology, Creighton University
Bevins, C., Human Genetics Branch, NICHD
Eizirik, D.L., and Sandler, S., Dept. of Medical Cell Biology,
  Uppsala University, Sweden

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-12 CI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Taste and Its Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Tylenda, Carolyn A.	Senior Staff Dentist	CIPC	NIDR

COOPERATING UNITS *(if any)*

LNS, NIA

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to elucidate the mechanisms by which oral sensory and perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. These methods, applied to the study of age-associated changes have provided insights into basic mechanisms of normal chemosensory perception. Functional variation under pathologic circumstances is assessed through objective evaluations of oral sensory disturbances occurring in association with systemic disease, salivary gland dysfunction, therapeutic x-irradiation or as an isolated complaint. Assessments of olfactory sensitivity are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE 00332-07 CI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberts, Michael W.	Dep Clin Dir NIDR/C	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Folio, John	Senior Staff Dentis	CIPC	NIDR
Shern, Roald J.	Senior Staff Dentis	CIPC	NIDR
Tylenda, Carolyn A.	Clinical Staff Dent	CIPC	NIDR
Wright, William E.	Senior Staff Dentis	CIPC	NIDR
Atkinson, Jane C.	Dental Staff Fellow	CIPC	NIDR
Kobel, Mary L.	Dental Hygienist	CIPC	NIDR

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, NIMH; Pediatric Branch NCI; Inter-Institute Genetics Program, CC; Arthritis and Rheumatism Branch, NIADDK; Letterman Army Institute of Research, San Francisco, CA; Diagnostic Systems Branch, NIDR

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 8.5	PROFESSIONAL: 3.05	OTHER: 5.45

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews, evaluation of various therapeutic regimens and roentgencephalometric analysis.

OTHER PROFESSIONAL PERSONNEL Z01 DE00332-07 CI

Lyons, Janet L.	Dental Hygienist	CIPC	NIDR
Hughes, Christopher V.	Dental Staff Fellow	CIPC	NIDR
Royce Leah S.	Dental Staff Fellow	CIPC	NIDR
Marini, Joan	Geneticist	CE	NCI
Mulvihill, John J.	Chief Clinical Genetics Section	CE	NCI
Guckes, Albert D.	Chief CODC	CODC	CC
Pizzo, Philip A.	Chief Pediatric Branch	PB	NCI
Termine, John J.	Chief, Bone Research Branch	BR	NIDR
Klippel, John H.	Senior Investigator	AR	NIADDK
Loe, Harald	Director NIDR	CIPC	NIDR
Cain, Janet L.	Volunteer	CIPC	NIDR
Katz, Ronald W.	Dental Staff Fellow	CIPC	NIDR
Ship, Jonathan A.	Dental Staff Fellow	CIPC	NIDR
Li, Shou-Hua	Statistician (Health)	EB	NIDR
Rudy, Susan F.	Clinical Nurse (General)	CC	NIDR
Webber, Richard L.	Chief Diagnostic Sys	DS	NIDR
Zeichner, Samuel J.	Senior Staff Fellow	DS	NIDR
Rinne, Norton F.	Volunteer	CIPC	NIDR
Moffa, Joseph P.	Chief Clinical Research Branch	Letterman Army Institute of Research	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00336-07 CI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Salivary gland secretion mechanisms during normal and altered functional states

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Ambudkar, Indu S.	Visiting Associate	CIPC NIDR
He, Xinjun	Visiting Fellow	CIPC NIDR
Horn, Valerie J.	NRSA Fellow	CIPC NIDR
Melvin, James E.	NRSA Fellow	CIPC NIDR
Wu, Xiaozai	Guest Researcher	CIPC NIDR

COOPERATING UNITS (if any)
LCMB, NIA

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.50	3.50	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize in vitro dispersed cells, and cultured cells of salivary glands, to understand mechanisms controlling saliva formation. We have focused these studies on autonomic neurotransmitter regulation of secretory events and associated signalling mechanisms. The aging rat parotid gland continues to be employed as a useful model to study autonomic receptor control of calcium handling in exocrine acinar cells. During this reporting period the primary focus of study has been directed at understanding the regulation of cytosolic calcium 2++ levels following α_1-adrenergic and muscarinic-cholinergic receptor stimulation. In particular, we have examined mechanisms of calcium 2++ release from intracelullar stores and extracellular entry of calcium 2++ via receptor- or second messenger-operated calcium 2++ channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00337-07

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Physiologicial Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fox, Philip C.	Dental Officer	CIPC	NIDR
Atkinson, Jane C.	Dental Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Caruso, Anthony	Staff Fellow	CIPC	NIDR
Larsen, Janne	Volunteer Worker	CIPC	NIDR
Macynski, Alice A.	Clinical Nurse	CIPC	NIDR
Moreira, Jorge E.	Visiting Associate	CIPC	NIDR

*See Additional Investigators

COOPERATING UNITS *(if any)*
RM, CC; DR, CC; HGB, NICHD; LIR, NIAID; MD, NIDDK; Columbia University; Boston University; SUNY, Stony Brook

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.85	5.40	2.45

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the function of various oral tissues during physiologic aging and in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness) utilizing the inpatient and outpatient services of the Dry Mouth Evaluation Clinic. Specific diagnostic approaches have been developed to aid in establishing the etiology of salivary gland dysfunction and defining criteria necessary for management decisions. A treatment protocol for selected patients continues, employing a regimen of oral administration of the parasympathomimetic drug, pilocarpine. Clinical and laboratory studies focusing on the etiology and character of the salivary gland component of Sjogren's sydrome, an autoimmune exocrinopathy, have advanced. An initial treatment protocol for primary Sjogren's sydrome has been started. In addition, detailed studies of salivary-associated oral complaints (eg. taste and oro-pharyngeal swallowing disorders) have continued. Such studies include evaluation of submandibular salivary and oral sensorimotor performance across the adult life-span in order to better understand dysfunctional states. Recent efforts have been directed to studies of oral mucosa; in identification of potential endogenous anti-microbial factors and alterations associated with salivary gland dysfunctions. A major effort has been instituted to characterize oral alterations associated with the acquired immune deficiency syndrome (AIDS). Studies focus on soft tissue changes, salivary gland function, and salivary-fungal interactions in AIDS, ARC and pre-AIDS (HIV-positive state). We have identified a salivary factor which is capable of inhibiting the infectivity of HIV-I for human peripheral blood lymphocytes.

Additional Investigators

Bevins, Charles	Medical Officer	HGB	NICHD
Lane, H. Clifford	Medical Officer	LIR	NIAID
Pillemer, Stanley	Senior Staff Fellow	MD	NIDDK
Ship, Jonathan	Dental Staff Fellow	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Tylenda, Carolyn	Dental Officer	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wolff, Andy	Visiting Fellow	CIPC	NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00411-03 CI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Health of Head and Neck Radiation Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wright, William E.	Senior Staff Dentist	CIPC	NIDR
Kobel, Mary L.	Dental Hygienist	CIPC	NIDR
Lyons, Janet L.	Dental Hygienist	CIPC	NIDR

COOPERATING UNITS *(if any)*
Pediatric Branch, NCI, and Radiation Oncology Branch, NCI

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.15	0.85

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies are evaluating the effectiveness of a formal orientation program designed to inform and motivate patients receiving head and neck radiation treatments using specialized oral health care regimens, and comparing preventive effectiveness of three topically applied fluoride regimens on the overall oral health status in the same population.

The subjects are divided such that a control group, orientated to the potential harmful oral side effects of radiation therapy by conventional verbal means, can be compared with a study group, oriented by a formal color slide-narration program developed at the NIDR dental clinic. In addition, individuals from each of the groups are randomly assigned in equal numbers to one of three oral fluoride regimens. A series of questionnaires and clinical diagnostic parameters are used to evaluate differences in patient compliance and the effectiveness of the therapeutic regimens as related to dental caries incidence and periodontal health status.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00412-03 CI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Maxillofacial Surgery and Implant-Prosthetic Reconstruction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Folio, John	Senior Staff Dentis	CIPC	NIDR
Roberts, Michael W.	Dep Clin Dir/NIDR/C	CIPC	NIDR
Wright, William E.	Senior Staff Dentis	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Guckes, Albert D.	Chief, CODC	CODC	CC
Gracely, Richard H.	Research Psychologist	NAB	NIDR
Li, Shou Hua	Statistician (Health)	EB	NIDR
Webber, Richard L.	Chief Diag Sy	DS	NIDR

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, CC; Nutrition Department, CC;
Surgical Services Department, CC; Diagnostic Systems Br., NIDR
Commissioned Officers Dental Clinic, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.05	1.55	2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evaluation of Rigid Versus Nonrigid Fixation Following Orthognathic Surgery

The purpose of this study is to determine the preferred method of fixation to avoid relapse following maxillary and mandibular osteotomy to correct facial developmental deformities. Correlations will be established, between rigid and nonrigid fixation techniques, and the degree of relapse as determined by radiographic, cephalometric and clinical assessment. Any changes in the height of the gingiva or the width of the attached gingiva will be recorded. Post-operative changes in facial contours and occlusion will be recorded. Pre and postoperative speech and swallowing will be assessed.

Clinical Study of Oral Endosseous Titanium Implants in Edentulous Subjects

The endosseous implant system consists of titanium root analogues with a threaded surface designed to be surgically embedded in the anterior third of the mandible. The root analogues are covered with a mucoperiosteal flap and the surgical site closed and allowed to heal. After healing, the root analogues are uncovered and coronal segment attached to each root analogue. A complete denture is constructed to restore the mandibular dentition. Cephalometric radiographs, the Cornell Medical Index, the Minnesota Multiphasic Personality Inventory, the Denture Satisfaction Questionnaire, a body focus questionnaire, a three day diet record and a rating of foods with respect to difficulty of chewing used to obtain data. The information obtained is utilized to determine if implant supported mandibular dentures significantly effect loss of verticle dimension of occlusion, satisfaction with dentures, food choices and nutrition, perception of difficulty of chewing selected foods, and body focus, when compared to treatment with coventional dentures.

OTHER PROFESSIONAL PERSONNEL

Rudy, Susan F.	Clinical Nurse (General)	CC	NIDR
Morgan, Vicror L., Jr.	Dental Laboratory Techn	CIPCB	NIDR
Stables, Gloria	Dietitian	NURT	CC
Sonies, Barbara C.	Speech Language Pathologist	RM	CC
Gaston, Gerald W.	Oral Surgeon (Consultant)	Univ. of MD-Baltimore	
Tilghman, Donald M.	Oral Surgeon (Consultant)	Univ. of MD-Baltimore	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00415-03 CI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ion Transport and Fluid Secretion in Salivary Glands

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Turner, Roy James	Visiting Scientist	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Manganel, Michel	Visiting Fellow	CIPC NIDR
Melvin, James E.	NRSA Fellow	CIPC NIDR
Corcelli, Angela	Guest Worker	CIPC NIDR
George, Janet N.	Chemist	CIPC NIDR
Lee, Syng I.	Guest Worker	CIPC NIDR
Busch, Kathryn	Guest Worker	CIPC NIDR

COOPERATING UNITS *(if any)*

Armed Forces Radiobiological Research Institute, Bethesda, MD

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Sections

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 5.7	PROFESSIONAL: 4.4	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Saliva is the principle protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations in the salivary secretory mechanism can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of primary fluid secretion by salivary acinar cells and thus to contribute to our understanding of the fluid secretory process in normal and disease states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad appicability and interest. During the present reporting period our specific areas of focus were the following.
(1) The transport of ions (sodium, potassium, chloride, bicarbonate) whose transmembrane and transepithelial movements are thought to be related to the process of primary salivary fluid secretion, was studied in vitro in a rat parotid acinar suspension and/or in isolated rat parotid basolateral membrane vesicles.
(2) The rabbit parotid Na/K/Cl contransporter was solubilized and partially purified using conventional protein separation procedures. The bumetanide binding site of this transporter was successfully reconstituted into liposomes. An essential sulfhydry group on the transporter was also characterized.
(3) Intracellular events (changes in calcium concentration and electrical potential) associated with muscarinic stimulation of individual parotid acini were studied using electrophysiological techniques and digital video imaging microscopy.
(4) Primary cultures of parotid acinar cells grown in defined media were studied in order to establish minimal requirements for cells viability, proliferation and differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00438-02 CI

PERIOD COVERED
October 1, 1986 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms Regulating Calcium Flux in Salivary Glands

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ambudkar, Indu S.	Visiting Associate	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Horn, Valerie	NRSA Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*
Department of Physiology, Johns Hopkins University, School of Medicine

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Sections

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	1.20	.40

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The secretion of saliva by the salivary glands is a complex processes which is regulated by neurotransmitters. Intracellular calcium mobilization, an early consequence of receptor activation, is a primary signalling mechanism in the regulation of salivary secretion. In order to understand the mechansims which regulate cytosolic calcium during the secretory events, we have directed our studies towards two areas (i) to characterize, at the cellular level, receptor-coupled signalling events leading to intracellular calcium mobilization and (ii) to characterize, at the membrane level, the systems which mediate the flux of calcium. In these in vitro studies we have utilized dispersed and permeabilized cells and isolated membrane vesicle preparations. We had previously shown that rat parotid basolateral membranes (BLM) possess an ATP-dependent calcium transport activity, which is modulated by membrane potential and potassium chloride flux and displays electrogenic characteristics in a reconstituted vesicle system. We had also shown that β-adrenergic receptor stimulation leads to intracellular calcium mobilization.

In the present reporting period we have shown that the BLM calcium transporter is regulated by calmodulin. We have assessed kinetically ATP-dependent calcium transporter activities in the endoplasmic reticulum and BLM, in relation to the cytosolic calcium levels achieved during stimulation. We have also shown that beta-2-adrenergic receptor activation, via cAMP, leads to inositol trisphosphate generation, a direct evidence of receptor "cross talk". We also have established that alpha-1-adrenergic and muscarinic-cholinergic stimulations result in mobilizing the same intracellular agonist-sensitive calcium pool. Finally, we have demonstrated that post-receptor effects on the beta-adrenergic signalling mechanism are induced by muscarinic receptor antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00455-01 CI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Candidiasis in AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Tylenda, Carolyn A.	Clinical Staff Dentist	CIPCB	NIDR
Yeh, Chih-Ko	Visiting Associate	CIPCB	NIDR
Larsen, Janne M.	Volunteer Researcher	CIPCB	NIDR
Fox, Philip C.	Dental Officer	CIPCB	NIDR

COOPERATING UNITS *(if any)*

Laboratory of Immunoregulation, NIAID

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.85	1.5	1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral candidiasis is one of the opportunistic infections commonly associated with AIDS. The overall objective of this work is to understand the relationship between the development of oral candidiasis and pathogenesis of AIDS. The initial study examined by culture the level of oral yeast in a population of HIV-I antibody positive AIDS patients with no history of opportunistic infections. Both the patient group and the normal control group consisted of non-smoking, non-denture-wearing males taking no medication. The mean level of yeast in the whole saliva of the AIDS patients was 13,000 colony-forming-units (cfu) per milliliter compared to a mean of less than 1.0 cf/ml in the control group. Indentification of the yeasts showed that Candida albicans was the predominant yeast in both groups.

These results indicate that 1) high oral yeast concentration in whole saliva may be an early sequela of HIV-I infection, 2) high levels of oral yeast precede overt clinical signs of candidiasis, and 3) the oral yeast in these patients results from proliferation of the normal yeast oral flora rather than colonization by unusual species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00457-01

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neurotransmitter Regulated Functions of Salivary Epithelial Cells in Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wellner, Robert B.	Senior Staff Fellow	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chief	CIPC NIDR
Ship, Jonathan A.	Clinical Staff Fellow	CIPC NIDR
Hand, Arthur R.	Dental Officer	CIPC NIDR
He, Xinjun	Visting Fellow	CIPC NIDR
Wu, Xiaozai	Guest Researcher	CIPC NIDR

COOPERATING UNITS *(if any)*
Dr. Mitsunobu Sato, Professor and Chairman,
Dept. of Oral and Maxillofacial Surgery, Tokushima University,
Tokushima, Japan.

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.5	0.7

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Saliva, the product of salivary gland secretion, is essential for the maintenance of oral health. Control of salivary secretion is mediated by the autonomic nervous system, which influences the ion, water and protein transport properties of salivary cells. In order to further our understanding of the mechanisms involved in the regulation of salivary gland secretion, we have sought to establish and study clonal lines of salivary cells which possess neurotransmitter signal transduction mechanisms coupled to functional cellular responses. The use of established cell lines offers several advantages over acute in vitro preparations: (1) long term studies can be carried out, (2) studies can be carried out on specific types of cloned cell lines which possess the same genetic background, and (3) somatic cell genetic and molecular biological techniques and be utilized to investigate various regulatory mechanisms. During this reporting period we have characterized several functional neurotransmitter responses in established rat human salivary epithelial cell lines in culture, and we have isolated several mutant cell lines which might be defective in processes subject to neurotransmitter regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00458-01

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
β-Adrenoreceptors and Gene Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kousvelari, Eleni	Senior Staff Fellow	CIPC	NIDR
Yeh, Chih-ko	Visiting Associate	CIPC	NIDR
Backman-Westman, Tuula	Guest Worker	CIPC	NIDR
Oliver, Constance	Senior Scientist	LI	NIDR
Siraganian, Reuben	Chf, Clin. Immun.	LI	NIDR

COOPERATING UNITS *(if any)*
ODDCBD,NCI; Roche Inst. of Molecular Biology; University of California, Primate Research Center

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.66	2.66	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cellular events in rat salivary glands are regulated by a variety of cell surface receptors. Specific stimulation of β-adrenoceptors in rat parotid acinar cells in vitro, increases levels of cAMP, protein production, phosphorylation and secretion. In vivo stimulation of rats by the β-adrenergic agonist isoproterenol results in hyperplastic and hypertrophic enlargement of the parotid and submandibular gland. In addition to these morphological changes there is an extremely high induction of transcription and synthesis of the proline rich proteins (PRP). We have shown that changes induced in parotid saliva protein composition after in vivo treatment of the animals with isoproterenol influence the adherence and aggregation of oral bacteria. In order to start understanding the mechanisms underlying the changes in parotid gland morphology and protein synthesis after β-adrenergic receptor stimulation, the expression of c-fos proto-oncogene, which encodes a nuclear protein which complexes with trans-acting factor, has been investigated. We have shown that c-fos expression was induced in rat parotid acinar cells after β-adrenergic receptor stimulation and this induction is specific since p53, c-myc, c-sis and c-abl were induced in a pattern different from that of c-fos. During this reporting period we have 1) localized the c-fos protein in the rat parotid cells; 2) demonstrated that c-fos gene expression is induced after stimulation of muscarinic receptors; 3) shown that c-fos induction is independent of the age of the animal; 4) observed increased c-fos expression in the RSMT-A5 epithelial cell line from submandibular gland after stimulation of β-adrenergic receptors; 5) investigated the effect of β-adrenoreceptor stimulation on c-abl gene rearrangement; 6) identified 4 clones which produce antibodies to different species of the PRP family and 7) been successful in maintaining parotid acinar cells in culture for approximately 90 days.

ANNUAL REPORT OF THE DIAGNOSTIC SYSTEMS BRANCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Diagnostic Systems Branch (DSB) is concerned with the isolation and identification of factors limiting performance obtainable from predominantly noninvasive diagnostic systems.

Particular emphasis is directed toward development of image-based systems designed primarily for dentistry, but the scope is broad enough to include research applicable to a variety of biomedical tasks.

In keeping with DSB's multidisciplinary approach to systems optimization, existing resources are deployed preferentially in collaboration with other agencies having common interests.

The task-specific systems focus of the Diagnostic Systems Branch, (DSB), is reflected in the description of continuing research activity into three general areas depending on the most significant factor limiting diagnostic performance. These areas correspond to: 1) Tasks limited by the data-sampling strategy i.e. so-called sample-limited systems, 2) Tasks limited by the amount of data acquired relative to the intrinsic variability of the observations i.e. so-called capacity-limited or noise-limited systems, and 3) Tasks limited by the ability of the system to appreciate or understand the diagnostic significance of the sampled data i.e. so-called interpretation-limited systems.

All continuing research efforts are encompassed by three broad project designations which evolved from early efforts to classify research according to the factors limiting performance as described above. These designations classify our research in terms of: 1) The enhancement of diagnostic images, 2) The development and evaluation of improved diagnostic systems, and 3) The exploration and assessment of new diagnostic modalities. The first designation deals primarily with methods for manipulating diagnostic data in specific, task-dependent ways; whereas the second is more global in scope, involving all aspects of data acquisition and interpretation essential to the diagnostic process. The third is relatively explicit, involving the sampling of new or unusual kinds of diagnostic information.

These efforts also represent extensions of a precedented commitment to design and produce a clinically useful, computer-based x-ray system having primary use and application in dentistry, and to develop new and more efficient methods for gathering diagnostic information. This commitment complements previous efforts to broaden the systems base of our research effort. To this end, it was decided to augment the system's focus by restructuring the distribution of research activity within the Branch such that more of the theoretical work would be done in house with the idea that it would be more productive to delegate a larger fraction of proposed clinical applications research to outside collaborators. This strategy permits more efficient use of constrained intramural space and resources while allowing more flexibility in the demonstration of practical utility applied to a broader range of clinical applications both dental and medical.

In keeping with this new philosophy, Dr. Dale Mirth, now with the Epidemiology and Oral Disease Prevention Branch, has agreed to serve as an interprogram project coordinator for a clinical trial of a new nonsteroidal anti-inflammatory drug, which incorporates state-of-the-art diagnostic techniques intended to demonstrate a new standard in the efficiency by which treatment effects can be elicited in clinical studies of this kind. This project conceptually parallels an ongoing multicenter field trial of this same anti-inflammatory drug which purportedly retards loss of alveolar bone in patients suffering from periodontal disease. The new investigation is designed to detect the effects of the drug much earlier than is likely to be possible in the larger multicenter study via the use of carefully controlled digital subtraction radiography. Additionally, periodontal pockets corresponding to regions demonstrating most severe bone loss are being surveyed for six different potentially pathogenic microflora to determine whether there is any obvious correlation of the presence of these specific bacteria with observed patterns of bone loss.

The sources of hardware support for such efforts also have been broadened significantly this year. In addition to the ongoing collaborative effort with the Radiation Physics Group at the National Bureau of Standards to build a tomosynthetic dental x-ray system, DSB has established a collaborative relationship with private industry to develop less expensive technology essential to the elimination of problems caused by lack of spatial registration intrinsic to existing methods underlying quantitative radiology. A particularly promising approach recruits a patient's own motor skills to aid in the positioning of the x-ray beam relative to the teeth. This is accomplished by having the patient view a closed-circuit television image of himself which intersperses a fixed image stored previously, with a real-time display on alternate frames. By moving his head such that the temporally alternating images superimpose spatially it is possible to minimize registration artifacts in radiographs exposed simultaneously.

Other progress in this area involves rejection of a first generation prototype x-ray tube having a finite number of focal spots which can be activated selectively under computer control, in favor of a new design which permits the generation of any number of focal spots which are more uniform when viewed over the complete range of angles required by the tomosynthetic process. At the same time, work continues in parallel to develop a practical high-performance intraoral x-ray detector. Research has shown that a hybrid design based on solid-state CCD technology appears to hold the most immediate promise at this time, and such a prototype chip has been fabricated for use in a miniaturized dental x-ray system being developed for military applications by the same group at NBS. A variation of this self-scanning device will be used to demonstrate feasibility in the complete tomosynthetic prototype currently under collaborative development by NIDR and NBS.

The ultimate goal of this work remains unchanged, and it is anticipated that the prototype emerging from this effort will have an even superior potential for synthesis of any desired x-ray projection from the radiographic information acquired during a single automated scanning sequence.

DSB has continued its systematic efforts to develop improved tomosynthetic algorithms which can be programmed into the prototype system once the transduction hardware is finally in place. This work has emphasized fundamental research being done primarily by Drs. Ruttimann and Qi involving the design of optimized spatial frequency filter kernels to minimize ringing artifacts intrinsic to the tomosynthetic reconstruction process. Published work in this area has demonstrated means for significantly reducing the need for time-consuming iterative algorithms to eliminate unwanted tomosynthetic blur.

Work continues on the implementation of a method for eliminating the present need for using a stent to stabilize projection geometry when generating radiographs intended for subtraction. The method is based on the theoretical realization that only one component of the registration error created by changes in projection geometry is irreversible. This irreversible component is determined by the spatial relationship existing between the x-ray source and the irradiated tissues of diagnostic interest. Earlier experiments done in collaboration with investigators at Harvard University confirmed the theoretical prediction that sagittal projections can be stabilized adequately with the aid of a conventional cephalometric x-ray positioning system in lieu of an occlusal stent. More recent work has centered on the design of a new type of cephalometric head holder that does not require the use of ear rods to aid in the positioning of the patient. Indeed, the new design requires no intrusive physical contact between the patient and x-ray machine at all. The prototype design described last year has been scrapped in favor of a simpler system which makes use of existing video-based technology developed by the RTS Corporation of Alachua Florida as described earlier with regard to the visually mediated x-ray registration system. Accordingly, all noncollaborative proprietary rights to this development effort originally held by the General Imaging Corporation now have been transferred to RTS, and this company has installed a working prototype device in the NIH Clinical Center for demonstration of clinical feasibility through application in an ongoing interdepartmental clinical trial.

Other research dealing primarily with diagnostic factors limited by sampling strategy is exemplified by the spectroscopic analysis of hemoglobin through differential attenuation of tooth-scattered light using a narrow band-pass optical filter. Progress on this project has been delayed because of technical problems resulting from the transfer of Dr. Maret Maxwell, the coprincipal investigator, from his Government appointment at BEIB into private industry. This action was complicated by the fact that the prototype system became damaged during renovation of BEIB facilities in Building 10, necessitating extensive rebuilding by technicians unfamiliar with its original design. A competent replacement for Dr. Maxwell has now been identified from recently recruited BEIB staff, and once the device is restored, a former guest worker in our laboratory, Dr. Arik Tzukert now at the Hebrew University, Hadassah School of Dental Medicine, Jerusalem, Israel, has agreed to do a comprehensive clinical evaluation of its efficacy in the objective measurement of degenerative changes in the dental pulp.

Particularly promising in terms of modality-specific applications is research based on the use of radiopharmaceuticals to predict loss of periodontal bone. Earlier findings suggested that the uptake ratio of 99mTc-MDP measured with a tiny cadmium telluride probe applied to active sites relative to control areas in a beagle dog model anticipated loss of supporting bone months before it could be detected radiographically. Recent work has involved efforts to use much more sensitive, quantitative methods to track bone loss radiographically combined with a more refined nuclear medicine technique in these animals. Careful measurements on a total of six dogs have now demonstrated a significant component of measurement variance attributable to the design of the prototype probe. We attribute this in part to a relatively broad acceptance angle, and are now experimenting with designs which are much more tightly collimated in order to reduce the opportunity for cross-arch "shine through" of the isotope. In this way, we expect to be able to improve the signal-to noise ratio of the device sufficiently to verify its potential as a diagnostic tool in dental applications. This work is being done in collaboration with Dr. Fasle Hosain, a Professor of Nuclear Medicine at the

University of Connecticut, who will be actively participating in this project as part of his planned sabbatical next year at NIH.

Another modality-specific project involves the use of magnetic resonance imaging (MRI) as a diagnostic probe to determine the degree to which stenosis of salivary ducts might be determined noninvasively. Controlled data derived from beagle dogs show conclusively that magnetic resonance images produced at selected intervals after stimulation of saliva by pilocarpine are easily distinguished depending on the degree of ductal stenosis involved.

Another investigation based on comparable images of the nasal mucosa in these animals has demonstrated the nasal cycle to be well approximated by a single sinusoidal control signal. The recently published results of this work have stimulated a substantial resurgence of interest in this unique physiological process by other groups outside NIH.

Research pertaining to capacity- or noise-limited systems is usually reflected in analyses of system efficiency. For example, the quantum yield of devices used to sample the distribution of photons produced by any radiographic or nuclear-medicine based system directly influences the exposure required to produce data with consistent statistical properties. Specifically, we have been exploring the effects of beam hardening caused by differential attenuation of the various irradiated tissues. Our first approach made use of a tissue equivalent attenuating ramp of known gradient and dimensions which was used to calibrate a series of fiducial wedges which were exposed simultaneously with the tissues of diagnostic interest. By matching tissue densities in the region of interest with a portion of the ramp which comparably filtered the beam overlying the image of one of the fiducial wedges it was possible to "tune" the system to an appropriate range of energies for purposes of densitometric calibration. This approach has since been supplanted by a more precise and accurate system based on the use of a special cassette which contains two sheets of nonscreen x-ray film separated by a calibration system comprised of multiple wedges of hydroxyapatite having known dimensions. On digital subtraction of the two exposed films, the resulting image of the calibration wedge system provides the computer with a look-up table which is necessarily preconditioned by any scatter and spectral hardening associated with passage of the primary beam through the tissues of diagnostic interest.

A third, theoretically related approach involves the recording of two radiographic projections simultaneously on films having differing x-ray energy sensitivities. This is accomplished by separating the two films with an attenuating filter and using emulsions having compensating speed characteristics. In theory this makes it possible to separate the two predominant energy-specific photon attenuation mechanisms involved; photo-electric, and Compton scattering. Extensive research done this year by a Visiting Fellow in DSB, Dr. Robert van den Berg, working in collaboration with scientists at the ADA Health Foundation has shown that this approach to dual-energy radiographic analysis is of limited practicality using existing nonscreen dental radiographic techniques. This is because passive filtration constrained by a reduction of exposure of a factor of two as required by the speed range of available nonscreen film emulsions is not adequate to separate the resulting energy spectra by the minimum required by theory to yield well-conditioned solutions to the underlying energy equations.

Interpretation-limited systems research perhaps is best exemplified by DSB's continuing effort to implement and augment quantitative methods for tracing changes in the size and shape of specific tissues over extended periods of time. Many useful algorithms have been developed and tested which facilitate the detection and measurement of lesions recorded in spatially registered images. Specific applications range from quantification of induced bone loss in the extremities, to tracing volumetric changes in bone attributable to periodontal disease or wound healing. This year DSB scientists have extended their focus to include arthritic applications. This work is being done in close collaboration with another NIH research group headed by Dr. Mark Bolander who is currently Chief of the Orthopedic Research Unit, NIAMS. Recent data confirm previous preliminary observations based on the use of cadaver material which show that known lesion volumes measured invasively and those deduced noninvasively from digital analysis correlate with an F squared of greater than 0.99.

Work also continues on the development of methods for automating the process of lesion detection. One aspect of this process involves the need to limit consideration to only the tissues of diagnostic interest. Recent efforts have focused on the development of an algorithm for detecting and delineating of areas in radiographs characterized by trabecular bone. An earlier approach used regional split and merge routines applied to data described with first order gray-level statistics in conjunction with quad-tree data management procedures. More recent efforts have been based on the application of fractal geometry to facilitate region characterization by fractal dimension. This work is being done in collaboration with Dr. Walter Kuklinski, at the University of Lowell in Massachusetts. The basic idea stems from the observation that trabecular bone patterns appear to be characterized by a reasonable degree of scale invariance which likely can be expressed as patterns constrained by a relatively constant fractal dimension.

Preliminary findings based on in vitro bone data conform to theoretical expectation and appear promising enough to warrant additional investigation next year.

Work continues in the collaborative research effort with physicians at Children's Hospital National Medical Center on the development of a physiologically based measure of severity of illness. New work has demonstrated applicability of this objective measure to assess quality of care provided in different intensive care units. In particular, it was shown that the intensive care given in tertiary health care centers compared to that in nontertiary care institutions is superior, if measured by severity of illness adjusted mortality rates.

Considerable effort continues to be expended in the transfer of technology developed earlier by DSB. Perhaps the most widely spread example involves the use of a digital method to compare periapical radiographs obtained from patients suffering from periodontal disease who are undergoing a variety of controlled treatment regimes. The technique involves registration of successively exposed films using a computer-based projection system and subsequent subtraction of stored images after correcting for contrast differences attributable to incidental changes in the x-ray exposure and processing chemistry. A new, more robust algorithm for quantifying osseous lesions has been devised. It is based on morphologic image-processing techniques which are insensitive to the shape of the lesion boundary. Controlled in vitro studies confirm that this approach is superior to the nodular-based method described previously.

Because DSB is a very small laboratory, research productivity is keyed to collaborative efforts with other groups both inside and outside the intramural program at NIH. The visibility afforded by these collaborations coupled with the significance of associated research has fostered a considerable demand for DSB investigators as methodological consultants in the areas of systems optimization and image processing. Recently this situation provided an opportunity for DSB to transfer some of its influence into a new department of “Diagnostic Sciences” at a major university. To this end, the current Branch Chief, DSB is planning to retire from government service to assume new responsibilities in academia on December 1. This move complements the strategy described above by providing room for upward mobility for scientific staff well-qualified in the theoretical science underlying diagnostic systems. It also provides a direct opportunity for continued collaborative research which promises to facilitate clinical application of technology developed by DSB over the last decade.

Publications:

Carcillo TA, Pollack MM, Ruttimann UE. et al Sequential physiologic interactions in pediatric cardiogenic and septic shock. Accepted for publication in Crit. Care Med.

Jeffcoat MK, Reddy MS, Webber RL, Williams RC, Ruttimann UE. Extraoral control of geometry for digital subtraction radiography. J Period Res 1987;22: 396-402.

Pollack MM, Getson PR, Ruttimann UE. et al Efficiency of intensive care - a comparative analysis of eight pediatric intensive care units. JAMA 1987;259: 1481-86.

Pollack MM, Katz RW, Ruttimann UE, Getson PR. Improving the outcome and efficiency of intensive care: The impact of an intensivist. Crit Care Med 1988; 165: 11-17.

Pollack MM, Ruttimann UE, Getson PR. The pediatric risk of mortality (PRISM) score. Accepted for Publication in Crit. Care Med.

Ruttimann, UE. Computer-based reconstruction and temporal subtraction of radiographs. Advances in Dental Resarch 1987;1: 72-9.

Ruttimann, UE, Qi XL, Webber RL. Optimal slice characterization in circular tomosynthesis. Proc SPIE Medical Imaging II 1988; 914: 728-35.

Ruttimann UE, Webber RL. Application of tomosynthesis in dental imaging. Proc SPSE Annual Conf 1988; 239-42.

Ruttimann UR, Webber RL.· Three-dimensional imaging in dental radiography. Proc 9th Annual Conf Engineering in Med and Bio Soc.1987; 735-36.

Ruttimann UE, Webber RL, van der Stelt PF. Automated registration and selection of radiographs suitable for subtraction. Proc 11th Annual Symposium on Computer Application in Medical Care 1987; 543-48.

Schmidt EF, Webber RL, Ruttimann UE, Loesche WJ. Effect of periodontal therapy on alveolar bone as measured by subtraction radiography. Accepted for Publication in J Periodontol.

Tsuchimochi M, Kato J. Evaluation of bone involvement by oral malignant tumors with scintigraphy of Tc-99m MDP and Ga-67 citrate. Japanese Journal of Oral and Maxillofacial Surgery 1987; 33: 2391-2407.

Webber RL. Factors limiting dental diagnosis: a conceptual overview. Advances in Dental Research 1987; 1:126-30.

Webber RL, Jeffcoat MK, Harman JT, Ruttimann UE. MRI evidence of simplicity and stability in the nasal cycle of a beagle dog. Journal of Computer Assisted Tomography 1987;11: 869-71.

Webber, RL. Some candid observations and reflections on the results of an in vitro test of the Siemens Periotest. Accepted for Publication in Compendium.

Welborn LG, DeSoto H, Hannallah RS, Fink R, Ruttimann UE, Boeckx, R. The use of caffeine in the control of post-anesthetic apnea in former premature infants. Anesthesiology 1988; 68: 796-98.

Welborn LG, Hannallah RS, McGill WA, Ruttimann UE, Hicks JM. Glucose concentrations for routine intravenous infusion in pediatric outpatient surgery. Anesthesiology 1987; 67: 139-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00065-17 DS

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development and Evaluation of Improved Diagnostic Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Webber, Richard L.	Chief, Diagnostic Systems	DS	NIDR
Ruttimann Urs E.	Bio-Medical Engineer	DS	NIDR
Zeichner, Samuel J.	Senior Staff Fellow	DS	NIDR
van den Berg, Harry R.	Visiting Fellow	DS	NIDR
Tsuchimochi, Makoto	Visiting Associate	DS	NIDR

COOPERATING UNITS *(if any)*
Childrens Hospital National Medical Center   RTS Corporation, Alachua, Florida
Radiation Physics Group, National Bureau of Standards
United States Army Institute for Dental Research, Washington, D. C.

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 2.64 | PROFESSIONAL: 1.61 | OTHER: 1.03

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects   ☒ (b) Human tissues   ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is an extension of previous work directed toward the study of noninvasive methods to determine spatial and temporal relationships existing between tissues of clinical interest. The approach involves in vitro modeling of promising systems and the development of prototypes suitable for clinical evaluation.

Recent work has focussed mostly on studies directed toward development of a versatile computerized dental radiographic system to produce images which can be subtracted to show small changes in tissue occurring over long intervals of time, and which can be combined in ways permitting synthesis of desired projections or tomosynthetic display of specific slices of the teeth and jaws.

Design principles of a new prototype x-ray tube have been established, permitting the generation of any number of focal spots on a planar target with the aid of a computer controlled electron beam. Specifications have been developed to build an intraoral x-ray detector based on solid state CCD technology, proximity coupled to a scintillation screen. Calibration methods and software are developed to control the effects of differential x-ray beam hardening in the quantitation of bone loss.

Research efforts continued on the design of a head holder system replacing the use of dental stents to stabilize the projection geometry in film-based radiography. Measurements on a prototype design demonstrated that the use of visual feedback by a closed circuit video system permits patients to position themselves reproducibly to enable subtraction radiography with sagittal as well as frontal projections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00211-12 DS

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enhancement and Processing of Diagnostic Images

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Webber, Richard L.	Chief, Diagnostic Systems	DS	NIDR
Ruttimann, Urs E.	Bio-Medical Engineer	DS	NIDR
van den Berg, Harry R.	Visiting Fellow	DS	NIDR
Qi, Xiang-lin	Visiting Associate	DS	NIDR

COOPERATING UNITS *(if any)*

Radiation Physics Group, National Bureau of Standards

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.11	2.08	1.03

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is an extension of previous work involving the creation, development and testing of image-processing techniques designed to improve diagnostic performance. Current work has centered on methods relevant to the processes of radiologic image subtraction and tomosynthesis. Work continues in the area of automatic manipulation to facilitate registration. Particular emphasis has been placed on: 1) methods for automatically segmenting radiographic images into regions of diagnostic interest, in anticipation of the automated detection of associated lesions, 2) methods for quantifying the apparent size of lesions from these images, 3) methods for increasing the efficiency of complex, spatial-frequency-dependent manipulations essential for optimization of diagnostic performance of specific tasks.

The recognition and delineation of areas showing trabecular bone was set as a primary target because of its importance in the diagnosis and monitoring of periodontal and other lytic bone diseases. Efforts continue in the use of second-order gray-level statistics for that purpose. Recent findings indicate that fractal geometry may provide a realistic model to characterize and segment trabecular bone.

Other work involves the use "shaded aperture" sampling techniques to eliminate ringing artifacts associated with high-pass filtered tomosynthetic reconstructions. Practical results show that optimum weighting of 25 projections permits substantial suppression of artifacts caused by structures lying in other planes..

Significant progress has been achieved in methods to quantitate lesion size. An automated procedure has been implemented to estimate bone mass loss that may be either focal or distributed over some area. The use of Fisher's linear discriminant function for detection threshold setting, followed by morphological image processing attains a precision 0.5 mg in dental and orthopedic applications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00373-06 DS

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Exploration and Assessment of New Diagnostic Modalities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Webber, Richard L.	Chief, Diagnostic Systems	DS	NIDR
Ruttimann, Urs E.	Bio-Medical Engineer	DS	NIDR
Zeichner, Samuel J.	Senior Staff Fellow	DS	NIDR
van den Berg, Harry R.	Visiting Fellow	DS	NIDR
Tsuchimochi, Makoto	Visiting Associate	DS	NIDR
Himel, Harvey N.	Physical Scientist	DS	NIDR

COOPERATING UNITS *(if any)*
Harvard School of Dental Medicine, Boston, MA
Biological Engineering and Instrumentation Branch, NIH
Hebrew University, Hadassah, Israel
Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.77	2.43	.34

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the evaluation of variety of new and existing diagnostic techniques which have yet to be applied in dentistry. It is largely a continuation of work done in previous years which dealt with the development of quantitative methods for measuring factors believed to be associated with relatively long-term changes of diagnostic interest in dental tissues. Modalities studied include quantitative radiology, nuclear medicine (99mTc-MDP), fiber-optic systems making use of visible light (hemoglobin-specific spectrum analysis), and magnetic resonance (MR) imaging.

New algorithms are being used to extend the application of digital subtraction radiography to include longitudinal assay of experimentally simulated, progressive, bone lesions in the phalanges and femur. Data show that gravimetrically determined focal lesions can be estimated with a precision of 0.5 mg.

Ratiometric assessment of scattered light continues to be used to evaluate tooth vitality. In vitro data obtained using a prototype, filtered, fiber-optic system designed to detect the presence of oxygenated blood in the pulp chamber show that tiny changes in tooth color measured in this way can be used to predict degenerative changes seen invariably in recently extracted teeth after storage for a week or more in saline at 37 degree C.

Measurements of differential uptake of 99mTc-MDP in beagle dogs following induction of tiny alveolar lesions indicated the need for redesign of the miniature nuclear probe to increase spatial specificity.

MR imaging was investigated to determine whether surgically induced stenosis of the salivary gland ducts can be detected reliably. The results indicate that T2-weighted images show significant differences between occuled and nonocculed ducts with excellent representation of the anatomy.

ANNUAL REPORT OF THE LABORATORY DEVELOPMENTAL BIOLOGY AND ANOMALIES NATIONAL INSTITUTE OF DENTAL RESEARCH 1987 - 1988

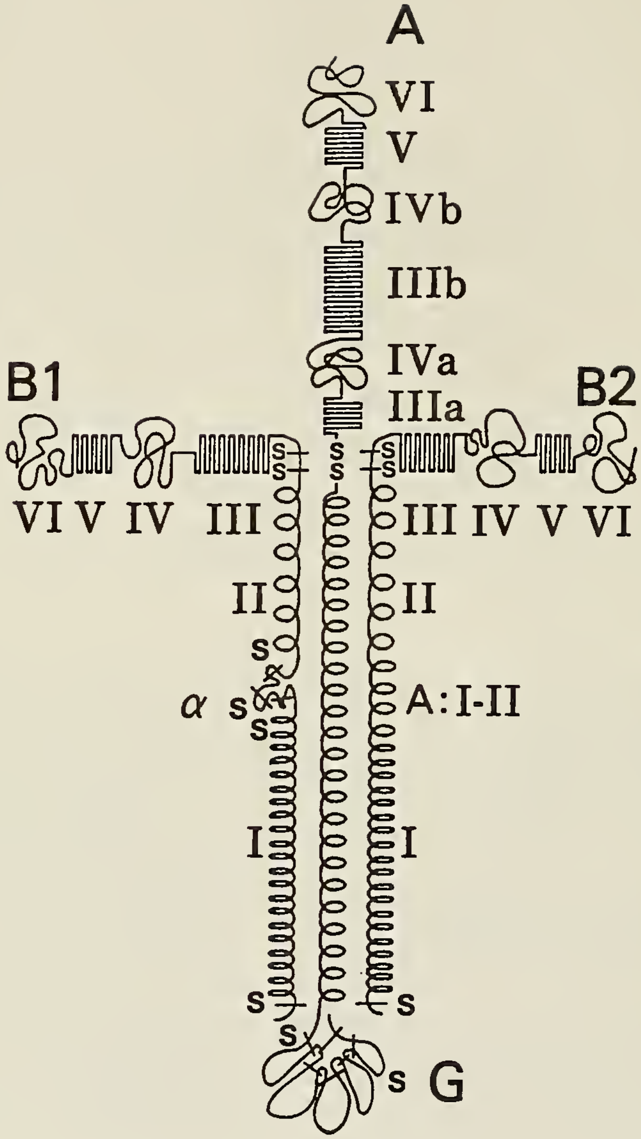
The Laboratory of Developmental Biology and Anomalies (LDBA) continues a dynamic program on the molecular and cellular role of extracellular matrix in development and disease. There have been a number of changes in the staff with the addition of two nephrologists Dr. Paul Klotman (Duke, on sabbatical leave) and Dr. Satoshi Horikoshi (Japan), and post-doctoral fellows including Dr. Beth Burrous (MIT) and Dr. Benjamin Weeks (University of Connecticut), Dr. Shun Kubota (Japan) and Dr. Hisae Hori (Japan). Several individuals in the LDBA have recently completed their assignments here. In addition, one of our senior scientists, Dr. John Hassell, has left NIH taking a professorship at the University of Pittsburgh. Dr. Hassell is an internationally known developmental biologist and expert on proteoglycans. While collaborative work will continue, we will clearly miss his daily contributions to this Laboratory. We have also been awarded the Doerenkamp-Zbinden Prize from the Johns Hopkins Center for Alternatives to Animal Testing for the development of an in vitro assay which measures tumor cell invasiveness and can be used to screen drugs, thus reducing the requirement for animal experimentation.

The Laboratory of Developmental Biology and Anomalies (LDBA) continues to emphasize molecular and cellular approaches to study the role of the extracellular matrix in development and in disease. Significant advances have been made in several areas. This is particularly evident in our studies on cartilage, on basement membrane, on wound and fracture healing, and on the invasive activities of tumor cells.

Basement membranes have been a major focus in this laboratory and several scientists are studying the structure, function and expression of its proteins and their cellular receptors. During the last year, we have finished the cloning and sequencing of the A chain of laminin. This work completes the primary structure of laminin, since we had sequenced the B1 and B2 chains previously. The structure of the laminin molecule deduced from this information is shown in figure 1 and indicates that the protein is composed of multiple domains. The sequence data have also been utilized to prepare synthetic peptides which exhibit some of the biological activity of laminin. The sequence YIGSR in the B1 chain serves as an attachment site for many cells. The RGD sequence in the A chain serves to support the attachment and differentiation of endothelial cells as well as the attachment of certain fibroblastic cells to laminin. Also progress has been made in identifying peptides from the A chain of laminin which stimulate the formation of axon-like processes by neural cells. It is possible that such synthetic peptides could be utilized in preparing biocompatible implants and grafts.

We have also discovered that the genes for the $\alpha1$ and $\alpha2$ chains of basement membrane collagen have a unique chromosomal arrangement and regulation. These genes are on opposite strands of chromosome 13 in a head to head arrangement separated by a 130 bp promoter. Expression of these genes is regulated by a common enhancer located in the first intron of the $\alpha1$ chain. Thus, a bidirectional promoter and a common enhancer coordinate and control the expression of these genes.

Fig. 1


A
VI
V
IVb
IIIb
IVa
IIIa
B1
B2
VI V IV III
III IV V VI
II
II
s
α s
s
A:I-II
I
I
s
s
s
s G

We have conducted parallel work on cartilage proteins and have, for example, cloned and sequenced the cartilage proteoglycan and link protein and studied their gene structure, chromosomal location, and involvement in cartilage diseases. Previously, we had isolated the promoter and enhancer of the major collagen (type II) in cartilage and showed by transfection experiments that both were required for the efficient transcription of this gene in culture. Now constructs have been synthesized which include the enhancer and promoter plus a reporter gene and these have been injected into fertilized mouse oocytes and lines of transgenic mice containing such constructs have been obtained. The reporter gene is only expressed in cartilaginous tissues of the transgenic mice and only when the gene was introduced in a construct containing both the enhancer and promoter. These results suggested that we could direct the expression of foreign genes specifically to cartilage. As a test of this system, we have prepared constructs of the enhancer and promoter coupled to the diphtheria toxin gene. Transgenic animals expressing this construct are found to have a variety of cartilaginous defects, cleft palate and the absence of eyes. Presumably the toxin gene is being transcribed in chondrocytes and is killing them. Subsequent studies will use such constructs as the promoter-enhancer plus interleukins or interferon to develop mouse disease models of inflammatory and weakened cartilage.

Considerable progress has been made in both developing and studying models of bone and skin healing. The healing of mandible and long bone fractures has been studied and shows strikingly different events in the two tissues. For example, no cartilagineous callus arises in the mandible fracture. Combinations of growth factors stimulate the rate of repair. Two new models of impaired skin healing have been developed. In one model, premature differentiation of epidermal cells is induced, thus blocking their coverage of the wound. This model may be relevant to chronic ulcers. The second model utilizes a drug which binds to growth factor receptors thus blocking the proliferative and chemotactic responses of the cells. This model may be useful in assessing the response of impaired wounds to exogenous growth promoting agents.

Studies on the invasive and metastatic activity of malignant tumor cells have become a major focus of LDBA research. This is the result of research here and in other laboratories that has shown that basement membranes are critical barriers to the spread of cancer. Many types of malignant tumor cells use common mechanisms to cross basement membranes including binding to laminin and producing collagenase IV to degrade the structure. The intra- and extracellular events have been defined in part. Prototypes of drugs have been identified that block these activities and convert malignant cells from an invasive to a dormant state. For example, we have shown together with collaborators at G.D. Searle that an inhibitor of collagenase IV blocks invasiveness in vitro and prevents the formation of tumors in vivo in mice. Such drugs could be useful adjuvants to surgery, radiation and chemotherapy in the treatment of cancer.

Introduction and Objectives

Interactions of cells with extracellular matrix components play an important role in the adhesion, migration, growth and differentiation of a variety of cells and influence tissue development and function. Our long range goals are to identify the molecular mechanisms which regulate the synthesis of extracellular matrix components and ultimately understand the role of the individual component during normal development and in pathologic states. We have been focusing on three areas: 1. the regulation of cartilage specific genes during chondrocyte differentiation; 2. the regulation and function of basement membrane constituents; 3. the structure and function of receptors for extracellular matrix proteins.

We are studying several model systems in which the synthesis of cartilage-specific proteins is modulated. For example, in chick chondrocytes, we are characterizing the mechanism by which expression of genes for these proteins can be manipulated in vitro, resulting in an expressed phenotype similar to that of fibroblasts. We are trying to define DNA sequences involved in the regulation of type II collagen gene and to isolate factors bound to the regulatory region of the gene. We have also isolated cDNA and genomic clones for link protein and for the large cartilage proteoglycan core protein.

Basement membranes have diverse functions including maintaining tissue organization and guiding tissue regeneration, in the filtration of macromolecule in cell adhesion and embryonic development. Basement membranes are altered in a variety of pathological states including diabetes, Alport's syndrome and glomerulonephritis. Futhermore, the basement membrane in contact with certain tumors becomes disorganized and allows the tumor cell to invade into normal tissue. Exploring the function of the basement membrane constituents and the regulation of their synthesis is essential for an understanding of basement membrane function. We have been studying gene structure and expression of type IV collagen and laminin in a variety of culture cells and tissues.

Method employed

(1) Introduction of segments of mammalian genes in the DNA of bacterial plasmids and bacteriophage; construction of recombinant DNAs; construction and screening of cDNA and gene libraries; (2) DNA transfection of mammalian cells, selection of transfected cells; (3) purification of specific DNA fragments; nucleotide sequence analysis of DNA and RNA; (4)measurement of RNA and protein synthesis in vivo and in cell-free systems; (5) expression of foreign genes in eukaryote and prokaryote cells; In Vitro mutagenesis; (6) purification of factors active in regulation of gene expression; (7) production of transgenic mice.

CARTILAGE

Cartilage Proteoglycan

We have completed cDNA cloning and sequencing of the rat cartilage proteoglycan core protein. Analysis of the sequence (2124 amino acids) suggests that the protein contains eight structural domains (Figure 2). There are two globular domains at the amino terminus, each with homology to link protein. Following these domains is a proline-rich segment thought to be the site of keratin-sulfate attachment, and then a large region rich in serine-glycine sequences which are likely to be the site for chondroitin-sulfate attachment. This region has distinct divisions based on the spacing of the serine and glycine sequences. Finally, the molecule terminates in another globular domain with homology to animal lectins and with the ability to bind carbohydrates such as fucose and galactose. We have defined the active region for the hyaluronic acid binding using expression constructs which produce the various segments of the amino terminal region of the core protein.

We have isolated the entire gene for the proteoglycan core from a rat genomic library. It is contained in eight clones spanning some 90 Kb of DNA and containing 16 exons which have been sequenced. The 5′ untranslated sequence is divided between two exons. There are 5 relatively small exons which encode the C-terminal globular domain, one large exon of 3.6 Kb encodes the entire chondroitin sulfate attachment region. The N-terminal globular domains are encoded by five exons. The exon sizes are completely conserved between the corresponding sequences of link protein and proteoglycan.

Kurt Doege, Yoshi Yamada

Link Protein

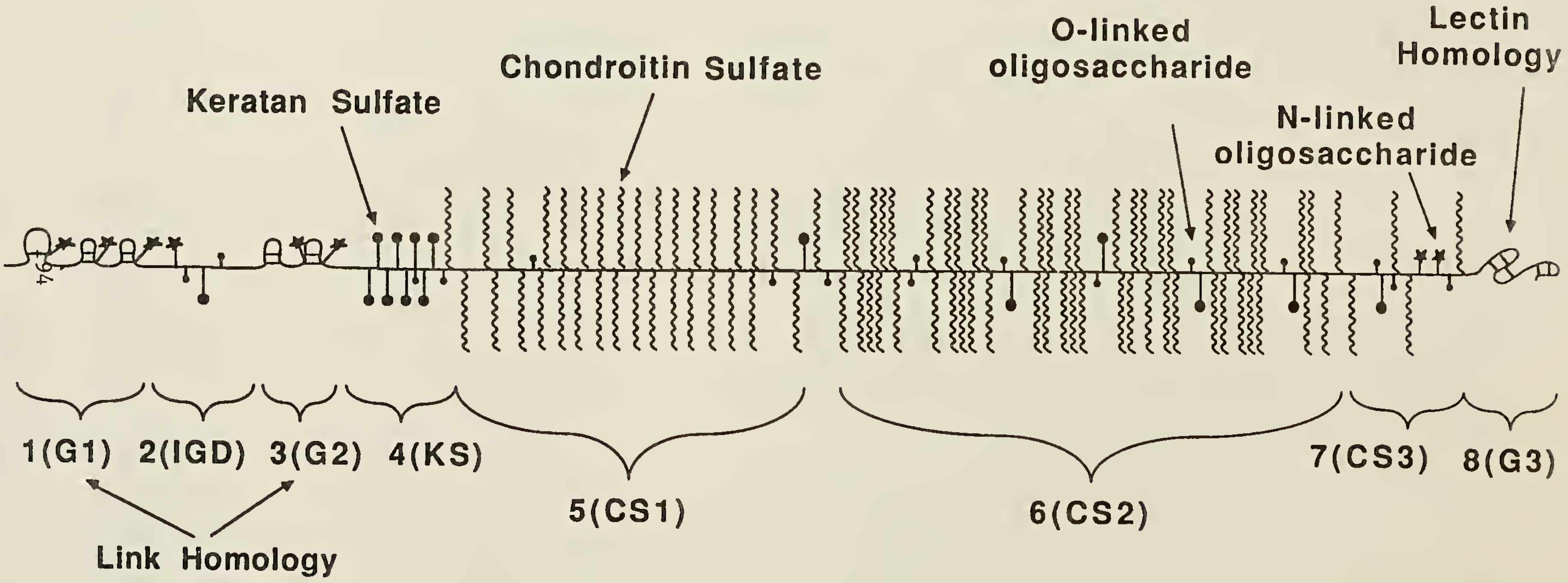
Multiple forms of link protein have been reported. We have found that alternative splicing allows a single gene to code for at least two different forms of link protein. Several overlapping genomic clones for rat link protein have been isolated and their exon structure has been determined by DNA sequencing. These results indicate that the exon structure corresponds well to the domain structure of the link protein. The complete sequence of the human link protein has also been determined by cDNA sequencing. Sequence comparison shows a strong sequence conservation between rat and human. We have mapped the link protein gene to human chromosome 5.

Craig Rhodes, Makoto Sasaki, Yoshi Yamada

Collagen II Gene

We have identified several cis-acting DNA elements in the rat collagen II gene which regulate the expression of the gene. One is an enhancer in the first intron of the gene which markedly increases the promoter's activity. We found that a 550 bp segment of the intron is required for maximum enhancer activity. Deletion analysis and gel retardation assays for the binding of

Cartilage Proteoglycan Structure



nuclear protein factors reveal that the enhancer contains multiple DNA elements which function synergistically. The enhancer is active in a position-independent manner and functions in either orientation. The enhancer shows activity only in cartilage and thus determines the transcription of the collagen II gene to these cells. It can stimulate the activity of a heterologous promoter, although less effective than the collagen II promoter. The nucleotide sequence of a 800 bp DNA segment containing the enhancer as determined. The sequence contains many direct and inverted repeats and several enhancer core sequences which are often found in enhancer regions of many genes.

There are three silencers, i.e. negative regulatory elements, which suppress the expression of the collagen II gene in fibroblasts. Two of them are present in the promoter region and the third is in the first intron of the gene. These silencers are not active in chondrocytes but function to block the transcription of the collagen II gene in non-chondrocytes cells.

The third regulatory DNA element is located at -100 to -56 from the transcription start site. This element is required for maximal transcription of the collagen II gene and it functions in conjunction with the other enhancer. Thus, the positive and negative regulatory elements play important roles in determining the levels of the expression of the collagen II gene.

Pierre Savagner, Tomo Miyashita, Koji Kanemoto, Yoshi Yamada.

Transgenic Lines

Transgenic lines have been produced using a 5'promotor and first intron enhancer element of the COL 2 gene with five different attached reporter genes. Chloramphenicol acetyl transferase (CAT), a bacterial gene that can be detected with high sensitivity provides a record of when and where the COL 2 gene is active. Using this construct in various forms we have shown that there is a high degree of variation in activity in different individual transgenic lines. However only cells known to produce type II collagen have shown activity in this system. The construct without the enhancer shows CAT activity only in the eye suggesting a different form of regulation for the type II collagen produced in the eye from that in the skeletal primordia. Chondrocytes in vivo seem to require the enhancer from the first intron for expression. Cartilage in the external ear of adults has CAT activity in some lines in which the skeletal cartilages have stopped being active. In general embryonic and newborn skeletal cartilages are much more active than those of weanlings or adults and the CAT activity of lines with 10 or more inserted copies of the construct is greater than those with few copies. It is not clear yet whether this is only a quantitative effect or is also a function of the location of the inserts in the genome since the lines with high activity also have multiple inserts segregating independently. However tissue specific activity is clearly a function of three variables, 1) The structure of the regulatory insert. 2) The number and site of the inserts, 3) the age of the animal.

The diphtheria toxin A gene stops protein synthesis in any cell in which it is activated, killing the cell in a specific manner. It has been attached to the

COL 2 promoter-enhancer construct. This has resulted in a number of transgenic fetuses with malformations of structures that are dependent on cartilagineous primordia and also produced a large number of deaths in early gestation at the time of major cartilage growth in early skeleton formation. These results suggest that the expression of the toxin gene is under control of the COL 2 promoter-enhancer in chrondrocytes.

Studies of regulation of COL 2 gene have been started that include production of transgenic lines containing the promoter-enhancer construct with several different genes that affect cell growth and function. These are SV40 T antigen gene, human C-FOS gene, TGFβ gene, and FGF gene. Basement membrane matrix molecule genes that have been introduced into transgenic mice include laminin B_1 and COL 4. Laminin B_1, gene appears to cause early embryo death when injected in large copy number. Only mosaic embryos or those with very low copy number survive. The transgenic approach has shown itself to be a very powerful tool for study of gene regulation in specific tissues. In addition to quantitative biochemical estimates of gene activity in tissues we have shown tissue activity by in situ hybridization using the CAT marker.

Kenneth Brown, Yoshi Yamada, Leslie Bruggeman, Xie Hou-Xiang.

BASEMENT MEMBRANE COMPONENTS

Laminin A Chain

Laminin, a major glycoprotein specific to basement membranes, is composed of three chains, A(400 Kd), B1 (220 Kd) and B2 (210 Kd). Previously we have determined the entire sequence of the B1 and B2 chain of mouse laminin. We now have completed the sequence of the A chain by cDNA sequencing. This spans 9520 bases and contains an open reading frame of 3084 amino acids including 24 amino acids of a signal peptide. The A chain contains some eight distinct domains with structural features for α-helix, cysteine-rich repeats and globules. There is considerable sequence and structural homology between the A chain and the B1 and B2 chains. However, the A chain has a unique globular structure at its carboxyl terminus containing homologous repeats and constituting one third of the molecular mass of the A chain. A sequence homology search indicates that the A chain shows homology to the Drosophila Notch, lin-12 in nematodes and the basement membrane heparan sulfate proteoglycan core protein.

Makoto Sasaki, Hynda Kleinman, Yoshi Yamada

Laminin B2 Chain Gene

The 5' portion of the mouse laminin B2 chain gene has been isolated and characterized by DNA sequencing. Two genomic clones which contain the first and second exons have been isolated. Exon 1 contains 641 bp including 229 bp of 5' untranslated segment, a sequence coding for the signal peptide and the N-terminal portion of the protein, while exon 2 contained 305 bp. The promoter region showed several interesting features including the presence of 9 "GC" boxes, a stretch of 9 nearly identical repeats of 11 nucleotides, and a sequence which is similar to the cAMP consensus sequence. Activity of the promoter of the B2 chain gene was tested by constructing B2-promoter-CAT plasmids and transfecting them into various cells. These studies showed that the B2 chain promoter is active in 3T3 cells and F9 cells.

Kohei Ogawa, Peter Burbelo, Yoshi Yamada

Expression of Laminin in Bacteria and Eukaryote Cells

We have expressed segments as well as the intact chains of laminin in bacteria and in eukaryote cells. β-galactosidase fusion proteins containing various segment of each chain were produced in bacteria using PEX vectors. These fusion proteins have been used to immunize rabbits to obtain domain-specific antibodies. Such proteins have been examined for their ability to bind to collagen IV and entactin/nidogen. We have also used a baculovirus vector for the expression of laminin chains in insect cells. One of the major advantages of this invertebrate virus expression vector over bacteria, yeast, and mammalian expression systems is the very abundant expression of recombinant proteins, which are produced in many cases, in a form antigenically, immunologically and functionally similar to their authentic counterpart. We have successfully expressed a cDNA encoding the entire B1 chain of mouse laminin with the yield of 20 mg per liter of culture. The partially purified B1 chain made in insect cells was able to stimulate cell attachment.

Kohei Ogawa, Makoto Sasaki, Yoshi Yamada

MATRIX RECEPTORS

Laminin Receptors

A variety of cells have high affinity binding sites for laminin and a specific laminin receptor (Mr=65- 69,000) has been identified. A partial human cDNA clone for this receptor has been reported. Using an oligonucleotide prepared from this clone as a probe, we have isolated mouse and human cDNA clones coding for the entire protein. The 3′ sequence of these clones corresponds to that reported earlier. However, our clone codes for a protein smaller (Mr=32,000) than the previously reported 67 Kd protein. The deduced amino acid sequence was highly conserved between mouse and human and lacked a typical transmembrane domain. Detergent extracts of solubilized cell membranes were found to contain both the 32 KD and the 67 KD species of laminin binding proteins and antibodies prepared against either the fusion protein deduced from the entire cDNA or the 67 KD molecule reacted with both the 32 KD and 67 KD proteins. Iodination of the cell surface components indicated that both proteins were associated with the cell surface. Antibody staining of these proteins was concentrated on the basal surface of polarized epithelial cells (MDCK) plated on a laminin substrate. Antibodies to the 32 KD protein reduced the invasiveness of human HT-1080 fibrosarcoma cells and inhibited the production of collagenase IV by the cells. These observations suggest that the two proteins are related and interact with laminin, and that their size difference may be due to differential splicing of mRNA or postranslational modifications of the proteins. Both the 32 Kd and the 67 Kd proteins are expressed by hepatocytes and a 3-fold increase is observed with hepatocytes in culture relative to the in vivo tissue.
Bartolome Segui-Real and Yoshi Yamada.

Receptors for Basement Membrane Proteoglycan Core

The basement membrane heparan sulfate proteoglycan consists of a large protein core with a compact elongated shape and three heparan sulfate chains attached to one end of the protein. Freshly isolated hepatocytes from normal adult rat liver bind and spread on the HSPG-core protein. The goal of this project is to identify the cell surface protein(s) that binds to the core protein of HSPG and to determine its biological significance. Following iodination of freshly isolated hepatocytes, plasma membrane proteins were purified, solubilized in non-ionic detergent, and then passed over a HSPG-core protein-Sepharose column. Bound proteins (Mr=38,000 and 34,000) were eluted with salt and another protein (Mr=26,000) was eluted with 8M urea. These data suggest that proteoglycan-cell association might involve the core protein and specific receptor(s). It is possible that the 38 and 34kD proteins serve as HSPG receptors and allow specific cellular responses.

Bruno Clement, George Martin, and Yoshi Yamada.

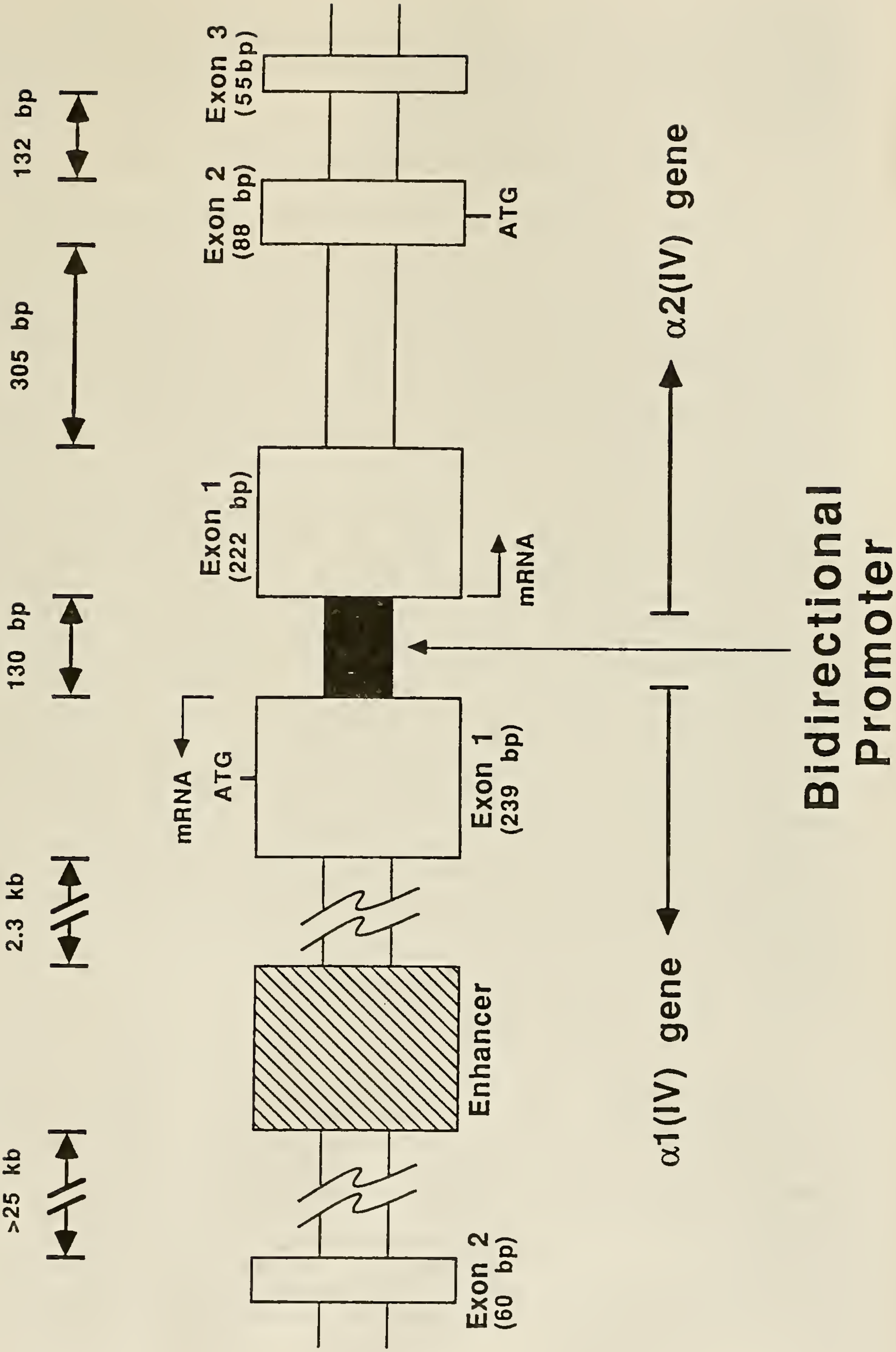
Collagen IV

We have isolated a gene encoding the entire mouse α1(IV) chain gene. It is more than 90 Kb in size and contains at least 34 exons. We have shown that the promoter of the gene requires for effective transcription an enhancer located in the first intron approximately 2.7 Kb downstream of the first intron. This sequence was defined as an enhancer based on its ability to stimulate transcription of the gene, independent of orientation and distance. The promoter of the α1(IV) chain gene showed it could function in either orientation with the enhancer. Further analysis revealed the presence of an opposite strand transcript, which was later identified as the α2(IV) chain gene. The α1(IV) and α2(IV) chain genes exist in a head to head arrangement separated by 130 bp of a bidirectional promoter as depicted in Figure 3. Transfection experiments suggest that the α1(IV) and α2(IV) genes are regulated by a common enhancer.

Peter Burbelo, Leslie Bruggeman, Paul Klotman, George Martin, Yoshi Yamada

Identification of Active Regions on Laminin for Neurite Outgrowth

Laminin is a potent stimulator of neurite outgrowth in both peripheral and central neurons. As little as 50ng/ml will cause cells to form processes two times the diameter of the cell body in one hour. We have sought to identify the active site in laminin for neurite outgrowth using proteolytic cleavage of laminin, chemical modification, and antibody inhibition. We have isolated an active pepsin-generated fragment which is composed primarily of the long arm. Antisera to synthetic peptides from the end of the long arm of the A and B1 chains were able to block laminin-mediated PC-12 cell neurite outgrowth whereas antibodies to other regions were inactive. Modification of lysine residues by either succinic or citraconic anhydride abolished laminin-mediated neurite outgrowth. Reversal of the citraconic anhydride reaction restored activity. Modification of arginine residues by cyclohexanedione has


>25 kb
2.3 kb
130 bp
305 bp
132 bp
Exon 2
(60 bp)
Enhancer
Exon 1
(239 bp)
ATG
mRNA
Exon 1
(222 bp)
mRNA
Exon 2
(88 bp)
ATG
Exon 3
(55bp)
α1(IV) gene
α2(IV) gene
Bidirectional
Promoter

no effect on neurite outgrowth but blocked cell attachment. Cleavage of methiones had no effect on neurite or cell attachment activity. We conclude that a specific neurite promoting site on laminin is contained within the long arm just above the A chain globule and that this site contains a lysine but not an arginine or a methionine residue.

Sephel, Tashiro, Ogawa, Yamada and Kleinman.

Identification of Biologically Active Sites on the Laminin A chain.

Previously we found an active 5 amino acid sequence (YIGSR) on the B1 chain (YIGSR) which promotes cell adhesion, migration, and 67 Kd receptor binding and blocks lung colonization by melanoma cells. We now find that this peptide in the cyclized form is much more potent in all of these assays. A nearby peptide PDSGR has some activity with the cells tested but is not as active as YIGSR. This peptide (PDSGR) appears distinct in laminin, competes with low relative activity for laminin in biological assays, and does not compete with fibronectin. Neither of these peptides is active for neurite outgrowth.

ARG-GLY-ASP (RGD) is contained within the A chain near the cross region and has been shown for many proteins including fibronectin, vitronectin, fibrinogen, etc. to be an active site for cell attachment. A variety of studies have also suggested that laminin can bind to the cell surface receptor for fibronectin and that antibody to this receptor can block laminin-mediated neurite outgrowth. We therefore sought to determine if this sequence from laminin was active and if the peptide could compete in laminin-mediated biological assays. We find that the RGD containing peptide from laminin CQAGTFALRGDNPQG is active for cell attachment, cell spreading, and cell migration. This peptide can also compete for laminin-mediated cell adhesion and migration. The peptide alone has no activity for the neurite outgrowth but when coupled to transferrin or to keyhole limpet hemocyanin it can stimulate neurite outgrowth with some cells. At concentrations as high as lmg/animal, this peptide (in contrast to YIGSR) was inactive in blocking melanoma lung cell colonization. Future studies are focusing on the cell surface receptor for this active site. Using cell surface iodination and peptide affinity column, a 75Kd "receptor" has been identified. We conclude that the RGD containing site is a biologically active site on laminin and that this activity may explain the attachment of some fibroblastic cells to laminin.

Tashiro, Sephel, Ogawa, Yamada, Kleinman.

A peptide located near the COOH-end of the A chain designated D12 (CSRARKQAASIKVAVSADR and the underlined sequences contained within it) adjacent to the terminal globule also contained very potent activity for cell attachment, and neurite outgrowth. All neuronal cells tested extended processes within one hour and the uncoupled peptide was active. This peptide was active in competition assays and appears to be the most potent peptide for a variety of cells. When tested *in vivo* to determine its role in the colonization of lungs by melanoma cells, it was found to cause an *increase* in the number of tumors formed. The increase was dose-dependent and was observable when the melanoma cells were injected in the vein and the peptide was injected intrapertonically.

An increase in collagenase activity is correlated with the metastic phenotype and peptide D12 has been found to increase collagenase activity to the same extent as laminin in HT-1080 cells. Current studies are aimed at identifying the active sequence within D12 and in determining the role of the peptide on tumor formation. Using all surface iodination and peptide affinity columns, a 63 kD "receptor" for the peptide has been identified on various cells.

Tashiro, Sephel, Ogawa, Reich, Burrous, Kleinman, and Yamada.

Mechanism of human umbilical vein endothelial cell tube formation

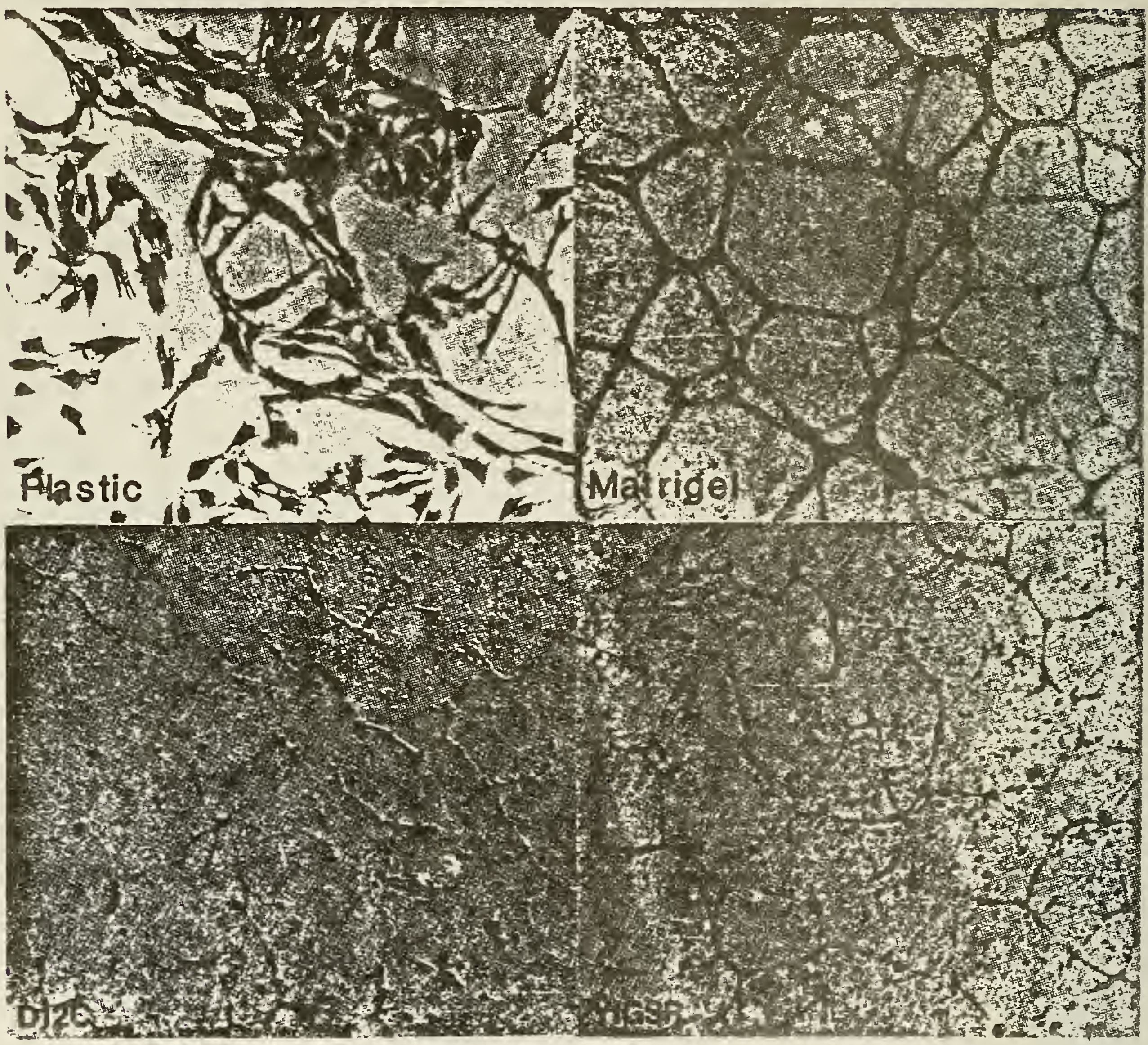
We have been investigating the process of angiogenesis in vitro using human umbilical vein endothelial cells. These cells rapidly (by 18 hours) form capillary-like structures when cultured on matrigel or on laminin supplemented matrices (Fig 4). The process of tube formation requires protein synthesis and in particular collagen synthesis since cycloheximide and cis hydroxyproline block tube formation. Ascorbic acid, a promoter of collagen synthesis, promotes tube formation. Furthermore, the cells bind rapidly to laminin and to surfaces coated with either YIGSR or the RGD containing peptide but D12 was inactive. Some synthetic peptides from laminin were found to inhibit tube formation. In particular, 200 to 500 ug/ml of YIGSR (especially in the cyclic form), and peptides containing RGD sequences were shown to inhibit 60% of tube formation. Peptide D12 caused the tubes to invade into the matrix. Our data suggest that multiple molecular events are involved in tube formation and our current studies are aimed at identifying the cellular receptors involved in the process.

Grant, Kleinman, and Martin.

Fig 4

differentiation

ENDOTHELIAL CELLS ——→ TUBE FORMATION



WOUND AND FRACTURE HEALING

Models for Defective Wound Healing

While defects in wound healing are common in elderly and in diabetic individuals, few models of defective wound healing have been developed in experimental animals. In evaluating the migration of keratinocytes in vitro, we noted that the cells showed chemotactic activity toward a number of growth factors (TGFα, PDGF, FGF) but when exposed to calcium ion they became immediately immobile. Application of calcium to experimental wounds strongly inhibited the coverage and contracture of the wound resulting in an open ulcer. Calcium blockers such as Verapamil, when added to the wound prior to the calcium, allowed near normal repair. It appears that calcium induces the premature differentiation of these cells. Also, it was noted that collagenase levels are highly elevated in the wound. We have also found that the application of surinam, a blocker of growth factor receptors to wounds, also impairs wound healing. The calcium model appears to be directed at the epidermal cells and surinam at the dermal cells. These models can be used to screen for pharmacological agents which might improve repair.This represents a model of defective wound healing resembling decubitus skin ulcers in some regards.

Tony Sank, George Martin

The Orthopaedic Research Unit which is part of NIAMS is located within LDBA and maintains a strong collaborative relationship with LDBA.

Gene Expression in Normal and Impaired Fracture Healing.

Remarkable cellular events are activated in a fractured bone that lead to the formation of callus and bone repair. These events include the migration of progenitor cells into the damaged area, proliferation and differentiation of these cells, and the initiation of extracellular matrix synthesis. Our intent is to identify the critical steps in fracture healing at the level of gene transcription. Using recombinant DNA technology we have found that a temporal sequence of gene activity is observable, and that this sequence is altered in models of abnormal healing, including diabetic animals and after adriamycin administration, and in fractures that heal by intermembranous bone formation.

Evaluation of healing fractures in diabetic animals demonstrated a severe defect in the development of mechanical strength. This was found to correlate with a 50% decrease in collagen content and a 40% decrease in DNA content.

Treatment with insulin at levels well below what is required to normalize blood glucose corrects this abnormality. Initial studies have demonstrated a decrease in the ratio of collagen I mRNA / total DNA in the diabetic callus, suggesting that the decrease in collagen content may be due to impaired gene expression. We are developing methods for organ culture of fracture callus, and will use these techniques to further investigate the expression of structural protein genes during fracture repair.

Type I collagenase (collagenase I) is known to mediate the remodeling of soft tissue during development and repair. Recent studies have demonstrated additional roles for collagenase, including degradation of the cartilaginous matrix in arthritis and regulation of osteoblast-mediated osteoclastic bone resorption. These data suggest that collagenase would be expressed during fracture repair and suggest that collagenase synthesis may mediate remodeling of the callus. Type IV collagenase (collagenase IV) degrades basement membrane collagen and is important in the remodeling of the basement membrane that occurs during capillary formation. Presumably, collagenase IV would be expressed during blood vessel formation and vascularization of the fracture callus. Numerous in-vivo studies have shown that inhibition of the cyclooxygenase or lipoxygenase branches of the arachidonic acid pathway, which lead to prostaglandin and leukotrine synthesis, decreases the synthesis of collagenase I and IV respectively. We would like to determine if these inhibitory effects on the arachidonic acid pathway can be shown to inhibit collagenase production in vivo, and if so, to define the role collagenase may have in fracture healing.

Studies to date have shown high levels of collagenase I and IV expression in the fracture callus between 4 and 7 days after fracture. Indomethacin, a drug which has been shown to have an adverse effect on fracture healing in animal models and to inhibit cyclooxygenase, PGE2 and collagenase synthesis _in vitro_, markedly increases PGE2 levels in the fracture callus. This data will be correlated with the direct measurement of collagenase activity at the fracture site. Finally, to better define the role of collagenase in fracture healing, mechanical strength and anti-collagenase immunohistology will be compared for fractures from different treatment groups.

The Expression of Proto-oncogenes and Heat-shock Protein Genes During Fracture Repair.

Several investigators have shown that cell-cycle dependent proteins (proto-oncogenes) and stress proteins (heat shock proteins) are expressed in some healing soft tissues. The conservation of both the nucleotide and amino acid sequences between species suggests a regulatory function for these proteins, and results form several studies support this hypothesis. Preliminary investigations in our lab have demonstrated expression of two heat shock proteins, HSP 89 and HSP 27, during fracture repair. Additionally, two proto-oncogenes, N-Ras and fos, demonstrate unique patterns of expression during healing, while no expression of myc, sis erb, or other proto-oncogenes can be detected. These observations may provide interesting parallels with other healing tissues and give insight into the regulation of cell differentiation in the healing callus.

The Role of Growth Factors in the Regulation of Fracture Repair.

Fracture initiates rapid, diverse and quite remarkable events which limit the loss of blood and initiate cellular migrations that lead to the restoration of the injured bone. Current concepts suggest that these cellular events are controlled in large part by growth factors, proteins which influence the proliferation and migration of cells. The sources of these growth factors would include platelets aggregated during clotting and the cells which subsequently populate the callus. Recent studies indicate that a number of growth factors exhibit both chemoattractant and mitogenic activity and could be functioning in a dual capacity in the callus. In fracture healing it seems clear that chemoattractants would be necessary to recruit cells into the callus, although this has not been shown directly. Presumably growth factors, as well as bone specific factors, may be necessary to induce progenitor cells to differentiate into cartilage and bone. We have initiated studies at both the molecular and protein level to identify growth factors in the callus. To date we have identified expression of TGF-B, IGF-2 but not IGF-1, and aFGF in the fracture callus. The correlation of growth factor expression with certain histological events and distinct changes in structural gene expression supports the hypothesis that these proteins have regulatory functions during fracture healing.

The Use of Polypeptide Growth Factors to Augment Impaired Fracture Healing.

Clinically, some 5% of fractures fail to heal normally, and repair is impaired in a variety of congenital and acquired conditions. We have noted that injection of aFGF into the callus can increase the rate of cartilage formation, while the infection of TGF-B can increase mesenchymal and fibrous tissue formation. We are currently evaluating the effect of exogenous growth factor administration in fracture healing. Impaired fracture healing may prove to be an important clinical condition where growth factors can be used to augment the repair process.
Gabor Nemeth, Ahlke Heydemann

INVASIVE ACTIVITIES OF TUMOR CELLS

Introduction

A classic sign of tumor malignancy is the invasion of the tumor cells through basement membranes into adjacent normal tissue. Basement membranes are sheet-like extracellular matrices which surround epithelial tissues as well as nerves, muscles and blood vessels. They are formed of collagen IV, laminin and heparan sulfate proteoglycan and serve as a physical support for various cells and tissues and also limit the passage of macromolecule and cells.

Passage of cells through basement membrane involves 1) binding of the cells to the basement membrane, 2) production of specific enzymes that degrade the basement membrane, 3) and movement of the cells into the normal tissue. We and others have developed a variety of assays to assess the ability of tumor cells to invade normal tissues and matrices. In our newest assay, we reconstitute an extract of basement membrane on a filter and measure the passage of tumor cells across the filter in response to a chemoattractant. Our studies show that metastatic cells readily cross this barrier while normal and benign tumor cells do not.

Studies on Metastatic Tumor Cells -

Malignant Tumor Cells Require Specific Arachidonic Acid Metabolites for Metastasis.

Previously, we developed a simple in vitro assay which measures the penetration of malignant tumor cells through basement membrane, a critical step in the dissemination of metastasis. Using this assay, we have found that malignant cells, when bound to the surface of the basement membrane produce and activate collagenase IV. This allows the penetration of the cells. We have found that inhibition of arachidonic acid metabolism via either the cyclooxygenase or lipoxygenase pathways prevents the production of collagenase IV and converts the malignant cells to a dormant state. Also we identified the critical metabolites of arachidonic acid as PGF12α and LTD4. Certain inhibitors of the lipoxygenase pathway suppress the invasiveness of human breast cancer cells, large cell lung cancer, and fibrosarcomas as well as the invasive and metastatic activity of mouse melanoma cells. It is possible that such drugs could serve as adjuvants to surgery, radiation and chemotherapy in the treatment of cancer. In related work, we have noted that malignant tumor cells treated in culture with eicosapentanoic acid, an analogue of arachidonic acid found in certain fish oils, also exhibit decreased invasiveness and metastatic activity. Possibly, such nutritional supplements could also slow the spread of cancer.

Reuven Reich, Erik Thompson, Leah Royce, George Martin

Studies on Small Cell Lung Cancer (SCLC) Cells -

Patients with small cell lung cancer have a poor prognosis as the cells metastasize widely and recur after chemotherapy. Cells cultured from these lesions proliferate rapidly in suspension and produce a variety of neuroendocrine factors. We have found that such cells attach readily to laminin in preference to other substrates. Attachment to laminin induces the classic form of SCLC to extend axonal-like processes as well as to produce collagenase. The so called variant form of SCLC attach to laminin but do not extend processes. Also, the classic cells are killed by a neural cell specific toxin (MPP) while the variant is resistant. Inhibitors of the lipoxygenase pathway of arachidonic acid metabolism down regulate laminin binding and cause the cells to detach from laminin substrates. These studies suggest that the SCLC cells are of neural lineage and that they show a high affinity for basement membranes. Possibly their ability to bind to laminin induces the cells to differentiate and become resistant to chemotherapy as their rate of proliferation is slowed.

Rafi Fridman, Tomoko Kanemoto, Reuven Reich, George Martin

Studies on Kaposi's Sarcoma (KS)

Kaposi's sarcoma is a highly vascularized tumor occurring in some 20-35% of patients with AIDS. There is uncertainty as to its origin and the nature of the cells comprising the tumor. We have studied cells derived from KS lesions and found them to be mesenchymal in nature and to show invasive activity comparable to other malignant tumor cells. Further, the KS cells secrete a factor that induces endothelial cell migration and invasion through basement membrane suggesting that the highly vascularized nature of the KS lesions is due to a strong angiogenic response by normal endothelial cells. We have also found that fibroblast growth factor (FGF) can also induce a similar response by endothelial cells.

An oncogene with homology to FGF has been isolated from KS lesions by

Basilico's group in New York. We have found that cells transfected with this oncogene also produce a potent angiogenic factor, although the cells are not invasive themselves. More recently, we have studied cells from transgenic mice developed by G. Jay (NCI) expressing the TAT gene of the HIV virus. These animals have abnormalities throughout their skin and develop KS-like lesions in some sites. Cells from these lesions also secrete an angiogenic factor. Such observations suggest that the TAT gene is activating FGF-like genes which in transformed cells induce the KS-like lesion.
Erik Thompson, Thomas Shima, George Martin with S. Nakamuro, Z. Sallahudin, NCI and G. Jay, NCI

Role of Oncogenes in Malignancy -

Cell transfected with certain oncogenes such as ras produce highly malignant tumors, To determine if this is a direct action of the oncogene, we are studying cells transfected with ras under the control of a dexamethasone-inducible promoter. Low levels of the steroid induce the transcription of ras and the synthesis of the p21 protein. Concomitantly, the cells become invasive and produce collagenase IV. This phenotype is fully reversed upon withdrawal of the steroid. These results indicate that ras directly elicits a malignant phenotype.

Rafi Fridman, Reuven Reich, George Martin

PUBLICATIONS OF FISCAL YEAR 1988

Adringer HH, Rose KM, Murray JE, Yamada Y. Hinc II and Kpu K RFLPs for laminin B1 (LAMB1) gene on chromosome 7. Nucleic Acid Res submitted.

Albini A, Gabriella A, Melchiori A, Giancotti F, Richtero H, Comoglio Paolo M, Parodi S, Martin GR, Tarone G. Chemotaxis of 3T3 and SV3T3 cells to fibronectin is mediated through the cell-attachment site in fibronectin and a fibronectin cell surface receptor. J Cell Biol 1987; 105: 1867-1872.

Albini A, Mitchell CD, Thompson EW, Seeman R, Martin GR, Wittek AE, Quinnan GV. Invasive activity and chemotactic response to growth factors by kaposi's sarcoma cells J Cell Bioch 1988; 36: 369-376.

Albini A, Auckerman SL, Melchinori A, Thompson EW, Reich R, Shima TB, Martin GR, Iwamoto Y. Basement Membranes: Reconstituted to assess the invasiveness of tumor cells. Proceedings of UCLA 1987; (in press).

Albini A, Aukerman SL, Ogle RC, Fridman R, Martin GR, Fidler IJ. The in vitro invasiveness and interactions with laminin of K 1735 melanoma cells. Evidence for different laminin-binding affinities in high and low metastatic variants. Clinic Exp Med 1988; submitted.

Aumailley M, Poschl E, Martin GR, Yamada Y, Muller PK. Low production of procollagen III by skin fibroblasts from patients with Ehlers-Danlos syndrome type IV is not caused by decreased levels of procollagen III mRNA. European J Clin Invest 1988; 18: 207-212.

Bolander ME, Young MF, Fisher LW, Termine JD, Yamada Y. Osteonectin cDNA sequencing reveals potential binding regions for calcium and hydroxyapatite, and shows homology with a basement membrane protein. Proc Natl Acad Sci USA 1988; 85: 2919-2923.

Bolander ME, Fisher LW, Rober PG, Prabhakar BS, Termine JT. Monoclonal antibodies against osteonectin show conservation of epitopes across species. Calcified Tissue International. 1988; (Submitted).

Brown KS, The Comparative Biology of Chondrodysplasias in Mice. Path and Immuno Path Res 1988; in press.

Brown KS. Hereditary defects of ectodermal derivatives: skin, teeth and ears. "Medical Genetics 1987", Course Syllabus (eds) Mulvihill J, Cameriui-Otero D, Schechter A, 1987; 346-357.

Burbelo P, Killen PD, Ebihara I, Sakurai Y, Yamada Y. Structure and Expression of Collagen IV Genes. Collagen: Biochemistry, Biotechnology, and Molecular Biology, Vol. IV Eds, M Nimni & B Olsen, CRC Press, in press.

Burbelo PD, Martin GR, Yamada Y. The $\alpha 1$ (IV) and $\alpha 2$ (IV) collagen genes are regulated by a bidirectional promoter and a shared enhancer, J Biol Chem

Campbell M, Horton W, Keeler R. Comparative effects of retinoic acid and jervine on chondrocyte differentiation. Teratology 1987; 36: 235-243.

Clement B, Segui-Real B, Hassell JR, Yamada Y, Martin GR. Identification of a cell surface binding protein for the core protein of basement membrane heparan sulfate proteoglycan. J Cell Biol (submitted).

Doege K, Sasaki M, Horigan E, Hassell JR, Yamada Y. Complete primary structure of the rat cartilage proteoglycan core protein deduced from cDNA clones. J Biol Chem 1987; 262: 17757-17767.

Ebihara I, Killen PD, Laurie GW, Huang T, Yamada Y, Martin GR, Brown KS. Altered mRNA Expression of Basement Membrane Components in a Murine Model of Polycystic Kidney Disease. Lab Inves 1988; 58: 262-269.

Emonard H, Grimaud JA, Peyrol S, Castronova V, Noel A, Lapiere CM, Kleinman HK, Foidart JM. Interactions between fibroblasts and a reconstituted basement membrane matrix. J Invest Dermatol 1987; 89: 156-163.

Fernandez PM, Selmin O, Martin GR, Yamada Y. The Structure of Anchorin CII, A Collagen Binding Protein Isolated from Chondrocyte Membrane J Biol Chem 1988; 263: 5921-5925.

Fridman R, Scott AF, Muller D, Reich R, Penno MB. Invasive and metastatic potential of murine adrenocortical carcinoma cells expressing different levels of normal c-ki ras proto-oncogene, Cancer Research, (submitted)

Graf J, Ogle RC, Robey FA, Sasaki M, Martin GR, Yamada Y, Kleinman HA. Pentapeptide from the Laminin Bl Chain Mediates Cell Adhesion and Binds the 67 KD Laminin Receptor. Biochemistry 1987; 26: 6896-6900.

Grant DS, Leblond CP, Kleinman H, Inoue S, Hassell JR. Laminin collagen IV and heparn sulfate proteoglycan assemble in vitro to form basement membrane-like structures. J Cell Biol, (submitted)

Halberg DF, Proulx G, Doege K, Yamada Y, Drickamer K. A segment of the cartilage proteoglycan core protein has lectin-like activity. J Biol Chem 1988; 263: 9486.

Horn VJ, Strum JM, Kleinman HK. Demonstration of Human Chondronectin in Fetal Cartilage and Loss with Exposure to Retinoic Acid Differentiation, (in press)

Horn V, Varner H, Dromsky G, Martin GR, Kleinman HK. Monoclonal antibody to human chondronectin In Development and Disease of Cartilage and Bone Matrix. (ed. Arup Sen, Thomas Thornhill) Alan R Liss Inc New York 1987; 265-273.

Horton WE, Miyashita T, Kohno K, Hassell JR, Yamada Y. Identification of a phenotype specific enhancer in the first intron of the rat collagen II gene. Proc Natl Acad Sci USA 1987; 84: 8864-8868.

Horton WG, Cleveland J, Rapp V, Nemeth G, Bolander M, Doege K, Yamada Y, Hassell JR. An established rat cell line expressing chardrocyte properties. Exp Cell Res 1988; (in press)

Horton WE, Yamada Y, Hassell JR. Retinoic acid rapidly reduces cartilage matrix synthesis by altering gene transcription in chondrocytes. Dev Biol 1987; 123: 508-516.

Horton WE, Yamada Y, Hassell JR. Retinoic acid - Induced alterations of chondrocyte gene expression: Implications for a teratogenic mechanism. Proceedings of the 8th CIIT conference on Toxicology. 1987; 215-228.

Howes R, Bowness JM, Grotendorst GR, Martin GR, Reddi AH. Platelet-Derived growth factor enhances demineralized bone martrix-induced cartilage and bone formation. Calc Tissue Int 1988; 42: 34-38.

Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y, Martin GR. YIGSR, a Synthetic Laminin Pentapeptide, Inhibits Experimental Metastasis Formation, Science 1987; 238: 1132-1134.

Iwamoto Y, Graf J, Sasaki M, Kleinman HK, Greatorex DR, Martin GR, Robey FA, Yamada Y. Synthetic Pentapeptide From the B1 Chain of Laminin Promotes B16F10 Melanoma Cell Migration. J Cell Phys 1988; 134: 287-291.

Iwamoto Y, Reich R, Nemeth G, Yamada Y, Martin GR. Cyclic AMP decreases chemotaxis invasiveness and metastatic potential of H-ras transformed mouse fibroblasts. Cancer Res (submitted).

Killen P, Burbelo P, Martin G, Yamada Y. Characterization of the Promoter for the α 1(IV) Collagen Gene: DNA Sequences within the First Intron Enhance Transcription. J Biol Chem (in press)

Killen P, Burbelo P, Sakurai Y, Yamada Y. Structure of the Amino-Terminal Portion of the Murine α1(IV) Collagen Chain and the Corresponding Region of the Gene. J Biol Chem 1988; 263: 8706-8709.

Killen P, Francomano CA, Yamada Y, Modi WS, O'Brien SJ. Partial structure of the human α2(IV) collagen chain and chromosomal localization of the gene (COL4A2). Human Genetics 1987; 77: 318-324.

Killen PD, Ebihara I, Laurie GW, Yamada Y, Martin GR, Brown KS. Abnormal expression of basement membrane genes in murine congential polycystic kidney disease. In: First International Symposium on Polycystic Kidney Disease 1987; in press.

Klein DJ, Brown DM, Oegema TR, Brenchley PE, Anderson JC, Dickinson MA, Horigan EA, Hassell JR. Glomerular Basement Membrane Proteoglycans are Derived From a Large Precursor. J Cell Biol 1987; 106: 963-970.

Kleinman HK, Ogle RC, Cannon FB, Little CC, Sweeney TM, Luckenbill-Edds L. Laminin receptors for neurite formation. Proc Natl Acad Sci USA 1987; 85: 1282-1286.

Kleinman HK, Graf J, Iwamoto Y, Kitten G, Ogle RC, Sasaki M, Yamada Y, Martin GR, Luckenbill-Edds L. Role of basement membranes in cell differentiation Cell Biol of Testis and Epididymis 1987; 513: 134-145.

Kleinman HK, Graf J, Iwamoto Y, Sasaki M, Schasteen CS, Yamada Y, Martin GR, Robey PA. Identification of a second active site in laminin for promotion of cell adhesion, migration, and inhibition of in vivo melonoma lung colonization. Biochemistry submitted.

Kubota Y, Kleinman HK, Martin GR, Lawley TJ. Role of laminin and basement membrane in the differentiation of human endothelial cells into capillary-like structures. J Cell Biol 1988; (submitted).

Laurent M, Martin GR, Sobel ME. Cell-free translation products of basement membrane RNA from the EHS tumor. Biochimica et Biophysica Acta 1987; 908: 241-250.

Laurie G, Inoue S, Bing J, Hassell JR. Visualization of the large heparan sulfate proteoglycan from basement membrane. Am J Anat 1988; 181: 320-326.

Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion. Proc Natl Acad Sci USA 1987; 84: 136-140.

Luckenbill-Edds L, Kleinman HK. Effect of Laminin and Cytoskeletal Agents on Neurite Formation by NG108-15 Cells. J Neur Sci Res 1988; 19: 219-229.

Martin GR, Timpl R. Laminin and other basement membrane components. Ann Rev Cell Biol 1987; 3: 57-85.

Martinet N, Harne LC, Grotendorst G. Identification and characterization of chemoattractants for epidermal cells. J Invest Derm 1988; 90:122-126.

Martinet N, Kim HC, Girard JE, Nigra TP, Strong DM, Chung SI, Folk JE. Epidermal and hair follicle transglutaminases: Partial characterization of soluble enzymes in newborn mouse skin. J Biol Chem 1988; 263: 4236-4241.

Martinet Y, Rom Grotendorst GR, Martin GR, Crystal RC. Exaggerated spontageous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. New England J Med 1987; 317: 202-209.

Miller RK, Brown K, Cordero J, Dayton D, Hordin B, Greene M, Guest Editorial, Position paper by the Teratology Soc. Vitamin A during pregnancy Teratogy 1987; 35: 267-268.

Miyashita Y, Horton W, Savagner P, Yamada Y. Analysis of a Cell Specific Enhancer in the First Intron of the Collagen II Gene: Determination of Boundaries and Nuclear Binding Factors. Mol Cell Biol

Nakanishi Y, Cuttitta F, Kasperzyk PG, Treston AM, Avis I, Minna JD, Kleinman HK, Mulshine JL. The effects of growth factors on the in vitro growth of small cell lung cancer as determined in a chlorimetric assay. In tumor: Lung Cancer Biology (Ed Rosen, Mulshine, Cuttitta, and Abrams) Marcel Dekker, NY, NY. 1988; (in press).

Nemeth GG, Bolander ME, Martin GR. Growth Factors and Their Role in Wound and Fracture Healing. Biol Clin Implications Alan R Liss NY pub 1988; 1-17.

Nemeth GG, Heydemann A, Bolander ME. Isolation and analysis of ribonucleic acids form bone and mineralized tissues. Analytical Biochemistry 1988; (submitted).

Noonan DM, Horigan E, Ledbetter S, Vogeli G, Sasaki M, Yamada Y, Hassell JR. Identification of cDNA clones encoding different domain of the basement membrane heparan sulfate proteoglycna J Biol Chem (in press).

Ogawa K, Burbelo PD, Sasaki M, Yamada Y. The Laminin B2 Chain Promoter Contains Unique Repeat Sequences and is Active in Transient Transfection. J Biol Chem 1988; 263: 8384-8389.

Oliver C, Waters JF, Tolbert CL, Kleinman HK. Growth of exocrine acinar cells on a reconstituted basement membrane gel. In Vitro 1987; 23: 465-473.

Pfaffle M, Ruggiero F, Hoffman H, Fernandez P, Selmin O, Yamada Y, Garron K, von der Mark K. Biosynthesis secretion and extracellular localization of anchorin CII, a collagen-binding protein of the calpactin family EMBO J 1988; in press.

Reich R, Stradford B, Klein K, Martin GR, Mueller RA, Fuller GC. Inhibitors of collagenase IV and cell adhesion reduce the invasive activity activity of malignant tumor cells CIBA Symposia Metastasis (in press).

Reich R, Thompson EW, Iwamoto Y, Martin GR, Deason JR, Fuller GG, Miskin R. Effects of inhibitors of plasminogen activator, serine proteinases, and collagenase IV on the invasion of basement membranes by metastatic cells. Cancer Research 1988; 48: 3307-3312.

Reich R, Martin GR. Identification of arachidonic acid pathways required for the invasive and metastatic activity of malignant cells. Cell (submitted).

Rhodes C, Doege K, Sasaki M, Yamada Y. Alternative splicing generates two different mRNA species of the rat link protein. J Biol Chem 1987; 263: 6063-6067.

Sasaki M, Yamada Y. The Laminin B2 Chain Has a Multidomain Structure Homologous to the Bl Chain, J Biol Chem 1987; 262: 17111-17117.

Sasaki M, Kleinman HK, Huber H, Deutzmann R, Yamada Y. Laminin, a multidomain protein: The A chain has a unique globular domain and is homologous with the basement membrane heparan proteoglycan and the laminin B chains, J Biol Chem (in press).

Segui-Real B, Savagner P, Reich R, Ogle RC, Martin GR, & Yamada Y. A 32 Kd cell surface-associated laminin binding protein: sequencing and characterization, EMBO, J (submitted).

Sephel GC, Burrous BA, Kleinman HK. Laminin neural activity and receptors. Devel Neurosci 1989; in press.

Sheutz EG, Wrigaton SA, Li D, Omiencski C, Eberhard VM, Kleinman HK, Elswick B, Guzelian P. Regulation and gene expression in adult rat hepatocytes cultured on an extracellular matrix. J Cell Physiol 1988; 134: 309-323.

Singer II, Scott S, Kawka DW, Hassell JR. Extracellular matrix fibers containing fibronectin and basement membrane heparan sulfate proteoglycan co-align with focal contracts and microfilament bundles in stationary fibroblasts. Exp Cell Res 1987; 173: 558-571.

Thompson EW, Elkington JSH, Blackshaw AW. Protoeglycan production by sertoli and myoid cells in mono-, co-, and parabiotic-culture Cell Biol of Testis Epididymis 1987; 513: 415-418.

Thompson E, Reich R, Shima T, Martin G, Dickson R, Lippman M, Albini A. Regulation of invasiveness of human breast cancer cell lines in vitro. Procd 3rd Inter Cong of hormones and cancer 1988; in press.

Thompson EW, Reich R, Martin GR, Albini A. Factors regulating basement membrane invasion by tumor cells. Cellular and Molecular Biology 1988; in press.

Thompson EW, Reich R, Shima TB, Albini A, Graf J, Martin GR, Dickson RB, Lippman ME. Differential Regulation of Growth and Invasiveness of MCF-7 Breast Cancer Cells by Antiestrogens. Cancer Research 1988; submitted.

Torchilin VP, Klibanov AL, Ivanov NN, Koteliansky VE, Gluckhova MA, Muzykantov VR, Martin G, Kleinman H. The components of the extracellular matrix as a target for targeted drug transport, In: Soviet Medical Revs. Cardiology, Vol 2: Ed. Smirnov VN, Katz AM. Harwood Academic Publ Chem Switzerland 1987; 550-560.

Weeks BS, Klein NW, Kleinman HK, Frederickson T, Sackett GP. Laminin immunized monkeys develop sera toxic to cultured rat embryos and fail to reproduce, Teratology in press.

Yamada Y, Graf J, Iwamoto Y, Kato S, Kleinman HK, Kohno K, Martin GR, Ogawa K, Sasaki M. Laminin: Structure, expression and cell binding sequence. In: The Cell in Contact II: Adhesion molecules in development and regeneration, 1988; in press.

Young MF, Bolander ME, Day AA, Ramis CI, Robey PG, Yamada Y, Termine JT. Osteonectin mRNA: distribution in normal and transformed cells. Nucleic Acid Research 1986; 14: 4483-4497.

Young MF, Findlay DM, Dominguez P, Burbelo PD, McQuillan C, Kopp JB, Robey PG, Termine JD. Osteonectin Promoter: DNA sequence analysis and S1 endonuclease site potentially associated with transcriptional activity in bone cells, J Biol Chem 1988; submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00009-27 DB

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Biosynthesis of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Martin, G.R.	Ch. Lab of Dev. Bio. and Anoms.	DB NIDR
OTHERS:	Reich, Reuven	Visiting Associate	DB NIDR
	Shima, Thomas	Guest Researcher	DB NIDR
	Yamada, Y.	Visiting Scientist	DB NIDR
	Laurie, G.	Visiting Associate	DB NIDR
	Fridman,Kanemoto,Sank	Guest Researchers	DB NIDR
	Chi, M.	Guest Researcher	DB NIDR
	Thompson, E.	Guest Researcher	DB NIDR

COOPERATING UNITS *(if any)*
Max-Planck Institute for Biochemistry, West Germany; McGill University, Canada; State University of New York at Buffalo; M.D. Anderson Hospital, San Francisco; Upjohn Cl, Kalamazoo, FDA, NCI.

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 8.90	PROFESSIONAL: 5.90	OTHER: 3.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the formation, and the destruction of connective tissue components in normal and diseased states. Particular attention is directed toward various matrix molecules including collagens, glycoproteins, and proteoglycans. Current aspects of this project include (1) the invasion of malignant cells through extracellular matrix, (2) alteration in the expression of matrix genes in development, disease and wound healing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00024-22 DB

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental processes in genetically controlled malfunction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brown, Kenneth, S	Medical Director, LDBA, NIDR	DB NIDR
	Bruggeman, Leslie	Post-Doc, LDBA, NIDR	DB NIDR
OTHERS:	Hori, Hisae	Guest Researcher	DB NIDR
	Klotman, Paul	Guest Researcher	DB NIDR
	Hou-Xiang, Xie	Visiting Fellow	DB NIDR

COOPERATING UNITS *(if any)*

Howard University, University of Maryland, University of Washington, Seattle; NCI, NIH, NEI, NIH; NIAMDD, NIH; and USDA Poison Plant Laboratory

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.00	7.50	4.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The availability of gene constructs for various extracellular matrix molecules, makes it now possible to test the function of these molecules, the regulation of expression, and use in gene replacement therapy by incorporating these constructs into mouse eggs, thus creating transgenic mice. A transgenic mouse facility has been established and staff has been trained to carry out timed breeding of donor and foster mothers. The complex techniques involved in DNA injection have been mastered and injections are carried out on a daily routine. The collagen II constructs in which various lengths of the 5′ flanking region are coupled to the gene for chloramphenicol acetyl transferase (CAT) with or without putative enhancer sequences have been used to produce a series of lines which differ in site of incorporation and copy number. Viable young which have incorporated the injected DNA into their chromosomes are then assayed for CAT activity to examine tissue specific expression. The BS-CAT construct (2Kb of the 5′ flanking DNA from rat collagen II gene fused to CAT) has been successfully established as a stable transgenic line but has not shown the expected tissue and time specificity variations but in general demonstrate the expected activity in tissues that contain type II collagen. We anticipate that this system will be useful in testing recombinant constructs of cartilage and basement membrane genes as possible models for gene therapy. When full length cartilage proteoglycans are available, we plan to use mutant (CDM) mice unable to produce cartilage specific proteoglycan as a test system. Other mutant mouse lines such as congenital and juvenile polycystic kidney disease, hypochondroplasia, chondrodysplasia, progressive ankylosis, and fragilitas ossium are potential targets for this type of genetic intervention when the molecular basis of their defects is determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00025-22 DB

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Connective Tissue Gene Expression During Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yamada, Yoshihiki	Chief, MBU	DB NIDR
	Martin, George	Chief	DB NIDR
	Doege Kurt	Staff Fellow	DB NIDR
OTHERS:	Sasaki, Makoto	Visiting Associate	DB NIDR
	Miyashita, Tomoyuki	Visiting Fellow	DB NIDR
	Rhodes, Craig	Biologist	DB NIDR

COOPERATING UNITS *(if any)*
Max-Planck Institut fur Biochemie; Johns Hopkins University; NCI; Roswell Park Memorial Institute; Sagami Chemical Institute; Columbia Univ.; Stanford University; FDA; NICHD

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION
Molecular Biology Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 12.00	PROFESSIONAL: 7.50	OTHER: 4.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to understand the molecular mechanisms by which genes for connective tissue proteins are regulated and expressed during normal development and in disease states. The formation of extracellular matrix is intimately associated with cell differentiation and tissue development in mammalian embryogenesis. These cellular responses are likely mediated through the specific interaction of a cell surface receptor with an individual extracellular components. Furthermore, alterations of extracellular matrix structure are associated with human conditions such as neoplastic disease and diabetes. We are studying genetic mechanisms controlling the expression and function of cartilage and basement membrane components as well as their receptors.

Recombinant DNA techniques have been used to prepare molecular clones and receptors of genes and mRNA for various cartilage and basement membrane constituents. Using these molecular tools, the genetic mechanism(s) controlling the expression of the genes has been studied. The structure of some of these constituents has been determined by cDNA sequencing. Relationships between the structure and function of the proteins have been studied using synthetic peptides deduced from cDNA sequence. Sequences in these genes regulating their expression are being studied.

Kleinman, Hynda	Chief, CBS, LDBA	DB NIDR
Ogawa, Kohei	Guest Researcher	DB NIDR
Hassell, John	Research Biologist	DB NIDR
Tashiro, Kenichiro	Visiting Fellow	DB NIDR
Burbelo, Peter	Staff Fellow	DB NIDR
Xie, Hou-Xiang	Visiting Fellow	DB NIDR
Savagner, Pierre	Visiting Fellow	DB NIDR
Bruggeman, Leslie	Postdoctoral Fellow	DB NIDR
Klotman, Paul	Guest Worker	DB NIDR
Koji, Kanemoto	Visiting Fellow	DB NIDR
Bartolome, Sequi-Real	Visiting Fellow	DB NIDR
Bruno, Clement	Visiting Fellow	DB NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00149-14 DB

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Proteoglycans During Abnormal Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:		
Hassell, John R.	Research Biologist	DB NIDR
Doege, Kurt	Staff Fellow	DB NIDR
Horigan, Elizabeth	Biologist	DB NIDR
OTHERS:		
Mosley, General	Bio. Lab. Technician	DB NIDR
Marks, Michelle	Postdoctoral Fellow	DB NIDR
Noonan, Douglas	Postdoctoral Fellow	DB NIDR

COOPERATING UNITS *(if any)*

Upjohn, Kalamazoo, Michigan (Steve Ledbetter), Saint Mary's Hospital, Manchester, ENGLAND (Paul Brenchley), Eli Lilly, Indianapolis, IN

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.20	1.20	2.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to determine the structure of proteoglycans as well as to understand the role that they play in the function of tissues and during developmental events. We have been using immunology, peptide mapping and molecular biology to characterize the core protein of proteoglycans from basement membrane. Basement membrane proteoglycans from EHS tissue consists of a MW = 4000,000 core protein 3 heparan sulfate side chains attached to one end. We have cloned and sequenced more than 60% (7Kb of our 12KB total) of the core protein. Distinct structural domains have been identified and should lead to more studies on function of this macromolecule. Using expression reactors containing certain regions of the cartilage proteoglycan, we have determined portion binds to hyaluronic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-12

PERIOD COVERED

October 1, 1987 - July 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proteins in Tissue Architecture and Cell Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kleinman, H.K.	Res. Chemist, Chief, Cell Biol. Sect.	DB NIDR
	Martin, G.R.	Chief, LDBA	DB NIDR
	Cannon, Frances	Biologist	DB NIDR
	Burrous, B.A.	Postdoctoral Fellow	DB NIDR
	Sephel, G.C.	Postdoctoral Fellow	DB NIDR
	Greatorex, D.	Biologist	DB NIDR
OTHER:	Grant, D.S.	Visiting Fellow	DB NIDR

COOPERATING UNITS *(if any)*

NCI, NHLBI, NIH, Max-Planck Inst. Biochem, Munich, Harvard Univ., Univ. of VA, University of Montreal and McGill University, Canada; Georgetown University; CNRS, Paris, Upjohn Co., Kalamazoo, MI; Univ. of Minn; Univ. of Wisconsin.

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 7.25	PROFESSIONAL: 5.50	OTHER: 1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most cells exist in tissues in contact or even surrounded by an extracellular matrix composed of collagens, glycoproteins, and proteoglycans. The interactions of the cells with the tissue-specific extracellular matrix components are important in the regulation of cell behavior. Our understanding of how the cells adhere to, grow on, migrate to, and differentiate on the extracellular matrix has been enhanced by the ability to purify the components and to test their biological effects both in vivo and in vitro. Specific molecules have been found which mediate the cellular responses both in matrix and on the cell surface. For example, laminin promotes the adhesion and growth of various epithelial cells while it appears to have a negative action of these activities for fibroblastic cells. We are currently investigating the biological activity of certain domains of the laminin molecule using synthetic peptides and domain and chain-specific antibodies. Specific adhesion and neurite promoting peptides have been identified which also disrupt endothelial cell differentiation. Our studies indicate that laminin has separate active sites for cell attachment and for neurite outgrowth. We are also determining the laminin receptors and tumor cell surfaces using molecular biological and biochemical approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00275-10 DB

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Testing of Fluoride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, George R.	Chief, Lab of Developmental Biology and Anomalies, NIDR
Brown, Kenneth S.	Medical Director, LDBA/NIDR

COOPERATING UNITS *(if any)*
University of Minnesota; NCI, NIH

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: .10
PROFESSIONAL: .10
OTHER: NA

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the action of fluoride in various biological tests used to detect clastogenic or mutagenic substances. To date fluoride has been examined in tests used to detect mutagens and found to be non-mutagenic. No effects on chromosome structure were noted in animals given widely different levels of fluoride. DNA repair after X-ray treatment was unchanged by fluoride. No genetic effects of fluoride were noted in a recessive lethal test of fluoride on Drosophila. The data indicate that fluoride has no mutagenic activity. Reports of fluoride's action on the metabolism and growth of cells are being monitored and where appropriate, related studies are undertaken.

The changes in major dibasic cation constituents of culture media produced by precipitation of insoluble fluoride salts does not increase mutagenesis in mouse lymphoma cells in culture.

Annual Report of the Laboratory of Immunology
National Institute of Dental Research
October 1, 1987 to September 30, 1988

During the past year, because of shifting fashions in intramural research at NIDR, the Laboratory of Microbiology and Immunology which has contributed consistently and meaningfully to the Institutes's mission for almost three decades was reorganized into two administratively separate Laboratories - Microbial Ecology and Immunology.

The Laboratory of Immunology will continue to perform basic and applied research on the contributions of mononuclear leukocytes, cytokines and microbial-host cell interactions to the development of chronic inflammatory diseases which include the acquired immune deficiency syndrome, rheumatoid arthritis and chronic periodontitis. Particularly noteworthy in this research has been the further elucidation of mechanisms of immunologically-mediated connective tissue catabolism, tissue repair and inflammation. In this regard and of considerable interest was the demonstration that Transforming Growth Factor Beta (TGF-ß) is a bifunctional regulator of the immune response. That is, TGF-ß has been shown to be a potent immunosuppressive agent on the one hand, while on the other it is a marked stimulator of fibrogenesis and angiogenesis. These findings indicate that TGF-ß may be beneficial in diminishing the tissue destructive consequences associated with lymphocyte-mediated chronic inflammation while promoting fibrosis and wound healing. Of significance also was the discovery this past year of differential control mechanisms that regulate lymphokine (Colony Stimulating Factors) gene expression in T and B lymphocytes. This information will add to the base of knowledge which is essential for the eventual modulation of the inflammatory response in a number of immunologically-mediated human diseases. In our programs on immediate hypersensitivity reactions, there is an increase emphasis on molecular biological approaches to define receptors on basophils and mast cells for Immunoglobulin E. Indeed, notable achievements have been forthcoming on the cloning of the IgE receptor and on the preparation of monoclonal antibodies to different epitopes on this receptor that inhibit antibody-mediated histamine release from basophils. These investigations have entailed numerous collaborative projects with other laboratories and institutions. Our accomplishments in these and other programs are documented below in greater detail.

The Cellular Immunology Section continues to explore the mechanisms of host defense to viruses, bacteria, protozoan parasites and other pathogens. These studies encompass mechanisms of cell recruitment, activation, proliferation and cytokine generation necessary to the development of cellular and humoral inflammatory reactions. Information obtained from cell culture systems and experimental animal models has provided the foundation for subsequent studies characterizing abnormalities in the network of cell-cell interactions which occur in diseases associated with immunodeficiencies and those associated with excessive immune function.

Central to the network of cellular interactions which constitute an immune response are the cytokines. Among these, the colony stimulating factors (CSFs) were originally defined by their ability to generate discrete colonies of specific maturing cells from hematopoietic progenitor cells of different lineages. However, more recent studies indicate that the CSFs are important immunomodulatory peptides. Our current research has shown that GM-CSF, which stimulates the proliferation and differentiation of progenitor cells, also stimulates the generation of reactive oxygen intermediates by circulating monocytes, resulting in enhanced cytotoxicity for microorganisms susceptible to oxygen-dependent cytotoxicity such as C. albicans. Furthermore, new observations indicate that GM-CSF also augments accessory cell function of circulating monocytes. Ongoing studies are addressing the role of class II molecules and IL-1 in GM-CSF augmented accessory cell function.

In an extension of these studies, investigators examined the effect of the various CSFs on the activation of monocytes as determined by the modulation of certain genes and the measurement of the translated polypeptide products. RNA preparations isolated from human peripheral blood monocytes cultured with varying concentrations of human recombinant G-, M-, GM-CSF and IL-3 were analyzed on Northern blots. When compared to monocytes cultured with endotoxin, G- and M-CSF generated relatively small amounts of mRNA for IL-1ß, IL-2R and small increases, if any, for HLA-DR. Functional and phenotypic changes correlated with the observed mRNA data. In contrast, GM-CSF and IL-3 were potent inducers of elevated mRNA levels for IL-1ß, IL-2R and HLA-DR. However, when the supernatants from such cultures were tested for IL-1, minimal biological activity was detected. This observation was unexpected since both GM-CSF and IL-3 induced mRNA at levels similar to that observed for endotoxin. However, RNA expression for IL1 is very transient which may account for the significantly reduced amounts of IL-1 bioactivity. Alternatively, a soluble inhibitor may act to neutralize IL-1 function or co-factor regulation may occur at a post-transcriptional level within the cell. Both GM-CSF and IL-3 also stimulated the appearance of mRNA for TNF-α demonstrating the important contribution of CSFs to the regulation of mature monocyte function.

In additional studies to understand the effect of CSF on myeloid cell differentiation, the murine myeloid leukemia cell line, M1, which can be induced to differentiate into mature macrophages was exposed to CSF. This process of differentiation which can be characterized by sequential expression of Fc and complement receptors (C3R), activation of specific enzymes, and morphological changes consistent with the transition from blast to mature cells can be induced by CSF. Interestingly, IL-1α alone induced the intermediate marker, lysozyme, but not the earlier expression of FcR and C3R or the morphological changes typical of terminal myeloid differentiation. However, when added to suboptimal concentrations CSF, IL-1α enhanced FcR and C3R expression as well as the morphological transition into mature cells. Consequently, IL-1α can induce M1 cells to become responsive to suboptimal concentrations of CSF and supports the concept that IL-1α is synonymous with hematopoietin 1.

Because of the important role that CSF plays in regulating not only proliferation of myeloid cells, but also in differentiation of monocytes, studies have also focused on regulation of CSF production and action. Cyclosporin A (CsA) is an immunosuppressant which blocks lymphokine synthesis by T cells at the level of mRNA transcription. In contrast, CsA does not inhibit monokine production by human monocytes after stimulation with CSF, nor does it block transcription of IL-1ß message in monocytes. Furthermore, GM-CSF appears to differ from other inducible lymphokines by not being inhibited in its synthesis by CsA. Studies to determine the mechanism of GM-CSF resistance to CsA demonstrated a posttranscriptional regulatory control site for GM-CSF production. Collectively, these observations indicate that GM-CSF production, as well as its ability to induce monocyte differentiation are not subject to control by CsA. This escape of GM-CSF dependent pathways from regulatory control by CsA may be key to the design and analysis of CsA therapy in transplant patients and in patients with rheumatoid arthritis undergoing immunosuppressive therapy.

Mononuclear cells generate additional hormone-like peptides with growth factor activity which are instrumental in the evolution and resolution of inflammatory reactions. TGF-ß, a product of platelets, T lymphocytes and monocytes appears to function as an inducing agent for other monocyte-derived growth hormones. Picomolar concentrations of TGF-ß stimulate peripheral blood monocytes to transcribe PDGF (c-sis), basic fibroblast growth factor (bFGF), interleukin 1 (IL-1), and tumor necrosis factorα (TNF-α). Each of these hormones exhibits a plethora of biological activities on other cell types, promoting inflammation as well as angiogenesis and fibrosis, favoring tissue repair. Recent studies have also demonstrated that while TGF-ß mRNA is expressed constitutively by human monocytes, TGF-ß protein is secreted only after activation of the monocytes, thus suggesting that separate control mechanisms regulate gene expression and secretion of this peptide. The observation that TGF-ß mRNA is synthesized constitutively while other growth factor genes are inducible suggested that TGF-ß may be involved in early events of monocyte activation. Inhibition of IL-1 mRNA levels by anti-TGF-ß antibodies in LPS-stimulated monocytes indicates that the increased IL-1 gene expression may actually be occurring through the direct action of newly-released TGF-ß. TGF-ß may be an important autocrine immunoregulatory molecule which plays a central role in the immune network.

Although TGF-ß induces IL-1 production, it is also a potent inhibitor of IL-1-dependent lymphocyte proliferation. TGF-ß at picomolar concentrations blocks the ability of IL-1 to stimulate lymphocyte proliferation. Inhibition of cell division appears to occur after activation of the lymphocytes inasmuch as neither gene expression nor translation of IL-2 receptors is suppressed. Furthermore, TGF-ß does not block synthesis of IL-2. Therefore, TGF-ß likely acts at a site distal to IL-1 to block lymphocyte DNA synthesis. These observations suggest that TGF-ß might provide a negative feedback mechanism to reverse the inflammatory response, yet enable IL-1, TNF and other monokines to continue modulation of tissue repair.

Suppressed lymphoid cell function is often observed in patients with chronic inflammatory diseases such as rheumatoid arthritis and leprosy. The mechanism of aberrant immune responsiveness associated with chronic inflammatory lesions is unknown, although adherent cells have been implicated. Because of the potential importance of impaired immunoregulation in the evolution of chronic inflammatory lesions such as arthritis, investigators in the Cellular Immunology Section have utilized the experimental model of bacterial cell wall induced chronic inflammation to explore the cellular and molecular pathways of suppressed immune responsiveness. Group A streptococcal cell wall (SCW) injected rats exhibit a profound immunosuppression which persists for months after the initial injection of SCW. When spleen cell preparations were depleted of adherent cells, restoration of T cell proliferative responses to Con A and PHA occurred, implicating adherent macrophages in the regulation of immunosuppression. Furthermore, macrophages from SCW-treated animals, when co-cultured with normal spleen cells in the presence of Con A or PHA, effectively inhibited the proliferative response. Supernatants from suppressed spleen cell cultures were also found to inhibit normal T cell mitogenesis. Taken together, these results implicated a soluble macrophage-derived suppressor factor in the down-regulation of T cell proliferation following exposure to SCW _in vivo_. Subsequent studies to identify this suppressor molecule(s) revealed the activity to be indistinguishable from TGF-ß. Furthermore, TGF-ß was identified by immunolocalization within the phagocytic cells of the spleens of SCW injected-animals. Interestingly, the mechanism of immunosuppression mediated by the SCW-activated phagocytic cells appeared to be similar to previous reports on the mechanism of TGF-ß inhibited mitogenesis _in vitro_. Although TGF-ß blocked DNA synthesis, it did not block the synthesis of lymphokines such as interleukin 2 nor the expression of IL-2 receptors on mitogen- activated T cells. These studies document that TGF-ß, previously shown to be a potent immunosuppressive agent _in vitro_, also effectively inhibits immune function in chronic inflammatory lesions _in vivo_. The unique ability of TGF-ß to augment certain aspects of the immune response while dampening others suggests that it may play an important immunoregulatory role in chronic inflammatory disorders.

Recent studies have focused on the influence of another cytokine, recombinant gamma interferon (γIFN) on the prostaglandin-cyclic AMP dependent pathway of collagenase production in monocytes. Initial studies demonstrated that γIFN inhibited monocyte collagenase synthesis as a result of its ability to block PGE_2 formation. More recently, γIFN has been shown to block the release of both cyclooxygenase and lipoxygenase products by interfering with phospholipase A_2 activity. These studies provide insight into the mechanism by which γIFN inhibits collagenase and prostaglandin production in monocytes and may be important in therapeutic modulation of chronic inflammatory lesions. To explore this possibility, γIFN was administered to bacterial cell wall-injected rats which develop chronic erosive polyarthritis. γIFN treatment resulted in clinically apparent suppression of arthritis. Although γIFN inhibited the acute response, it had a greater suppressive effect on the chronic, mononuclear cell-mediated destructive phase of the disease. Histologic examination of the synovium demonstrated that γIFN-treated animals had significantly fewer inflammatory cells, less synovial hyperplasia, and fewer erosions than the SCW controls. These data clearly demonstrate that the pathophysiology of SCW-induced erosive polyarthritis is subject to regulatory control by γIFN. This anti-arthritic effect of γIFN may be the consequence of its ability to inhibit PGE_2 and collagenase synthesis, block monocyte recruitment and/or reduce synovial proliferation. Ongoing studies will continue to define the mechanisms whereby γIFN inhibits the arthritic lesions because of its importance in current clinical trials in human rheumatoid arthritis.

Whereas an overactive monocyte-macrophage response in chronic inflammatory lesions such as arthritis can have pathologic consequences, impaired or deficient monocyte function can also be detrimental to the host. HIV-1 infection leads to impairment of numerous immunological parameters characteristic of the acquired immune deficiency syndrome. The immunosuppression is frequently manifested as susceptibility of the host to a variety of opportunistic infections. In order to determine whether HIV-1 infection might directly influence the capacity of monocytes to migrate to inflammatory sites, or whether deficient chemotactic function identified in AIDS patients is a secondary event, purified human peripheral blood monocyte populations were exposed to a macrophage-derived strain of HIV-1 _in vitro_ and the impact of that interaction on monocyte chemotactic ligand receptor expression and on directed migration was evaluated. Normal monocytes infected with HIV-1 or exposed to the HIV-1 envelope glycoprotein gp120 _in vitro_ exhibited a reduction in FMLP and C5a receptors which was associated with chemotactic dysfunction to these stimuli. Thus, the initial encounter of HIV-1 with the monocyte might trigger the cells to modulate their chemotactic ligand receptors. Such an interaction with circulating virus or free gp120 may help to explain how monocytes, even though not harboring detectable virus, might be rendered nonresponsive to chemotactic stimuli. In addition, the presence of shed gp120 early in infection may provide a mechanism for monocyte chemotactic suppression even in ARC patients.

Further investigation concerning the gp120-CD4 receptor interaction revealed that gp120 induced the production of IL 1 and arachidonic acid metabolites from the cyclooxygenase and lipoxygenase pathways. Unlike native gp120, the recombinant, non-glycosylated gp120 fragments, PB1-RF and PB1-IIIB, or p24, one of the core structural proteins of HIV-1, did not increase arachidonic acid metabolism, IL-1 activity, or the steady state levels of IL-1 mRNA. Similar changes in monocyte function and receptors can also be observed after the initial exposure of these cells to HIV-1. These findings demonstrate that soluble gp120 or gp120 on the virion is directly involved in the transduction of a signal, possibly through the OKT4A epitope on the CD4 receptor, which affects monocyte function and as a result may influence immune function.

Abnormal regulation of cellular genes may also play an important role in the pathogenesis of AIDS. To determine the effect of HIV-1 infection on monocyte function and phenotype, the expression of various growth factor and cell surface marker genes was analyzed after viral infection. RNA was prepared from HIV-1 exposed and control cells at various time intervals from 2 to 24 hours and analyzed by Northern blot hybridization. The HIV-1 exposed cells consistently demonstrated an early increase (2-6 hrs) in steady-state levels of IL-1 and IL-2R mRNA over that of control cells. Upon further activation of these cells with endotoxin, no difference in growth factor or cell surface antigen RNA levels was observed between control and HIV-cells.

At later stages of infection, dramatic differences in growth factor and cell surface antigen expression were observed in fully-infected monocytes. Spontaneous and endotoxin-induced levels of cytokines, TNF-α , IL-1, TGF-ß, and GM-CSF RNAs were elevated in HIV-1-infected cells between 7 and 14 days, the time during which viral replication was rapidly increasing. On the other hand, cell surface marker RNAs including IL-2R, HLA-DR, and T4 were dramatically diminished with the onset of viral replication. The excessive production of certain growth factors may contribute to the pathogenesis of some manifestations of AIDS. In addition, the decreased expression of cell surface antigens may alter the cell-cell communication important for mounting an immune response and thus contribute to the immuno-depressed state. It is not yet known whether the changes in gene expression in HIV-1 infected monocytes are the result of a host response to the virus or the byproduct of viral activation of cellular genes. Future work in collaboration with the FDA and the National Cancer Institute will involve the description of HIV-1-induced nuclear binding proteins in monocytes and the effect of these proteins on monokine and HIV expression.

In addition to circulating monocytes, tissue macrophages have also been examined in AIDS patients. Large numbers of macrophages containing undegraded infectious pathogens such as M. avium-intracellulare, Histoplasma capsulatum, and Cryptococcus neoformans have been identified, suggesting that in vivo, HIV-1 may impair the ability of macrophages to process certain microorganisms. Prompted by these observations, monocytes were exposed in vitro to HIV-1 and shown to spontaneously produce increased levels of O^-_2, likely due initially to gp120 activation of the cells via interaction with surface CD4, and subsequently to activation by virus replicating within cytoplasmic vacuoles. In contrast, coinciding with the appearance of replicating virus, the monocytes become refractory to secondary signals and cannot be induced to secrete toxic oxygen radicals that mediate oxygen-dependent cytotoxicity. The reduction in stimulated O^-_2 production correlates with a reduction in monocyte cytotoxic activity for C. albicans. Thus, the increased spontaneous, but defective stimulated, secretion of O^-_2, the precursor for other toxic oxygen radicals, may contribute to tissue injury, such as subacute encephalitis and nonspecific pneumonitis, and to increased susceptibility to opportunistic pathogens.

The most important pathogen in the oral cavity in AIDS patients is C. albicans. In preliminary findings, the Candida among 17 AIDS patients and 17 control subjects were shown to be similar by DNA "fingerprinting" implicating a defective host immune response to Candida, as opposed to an unusual biotype, in the pathogenesis of the mucosal candidiasis of the oral cavity and esophagus in AIDS patients. Since the immune response appears to be the decisive factor in the clinical manifestations of this pathogen, efforts continue to focus on monocyte killing of Candida. Using a newly developed assay for measuring monocyte cytotoxicity for the yeast, studies will explore whether HIV-1 mediated alterations in Fc receptors impair monocyte killing of this particularly relevant pathogen.

The important new observations described above regarding the effect of GM-CSF on monocyte maturation and function appear to have direct clinical application to the monocyte abnormalities in AIDS. In recent collaborative studies, recombinant GM-CSF administered to these patients has been shown to enhance monocyte O^-_2 production and HLA-DR expression. Thus, GM-CSF may be clinically useful in augmenting monocyte effector and accessory cell function in AIDS patients, in addition to its beneficial effects on myelopoiesis.

The Clinical Immunology Section studies the mechanisms involved in secretion from cells. Cells used in these studies include mast cells/basophils and pancreatic acinar cells. Cultured basophilic leukemia cells are very useful for biochemical and morphological studies to understand the mechanisms involved in the activation of the cells and the release of cellular granules. These cells divide rapidly in culture, have granules containing histamine and serotonin, grow attached to plastic and have surface IgG and IgE receptors. The cells are activated by crosslinking of the immunoglobulin receptors on the cell surface to release their granules. The release of mediators from mast cells/basophils is a model for cell secretion. These cells secrete two types of mediators, the preformed mediators (e.g. histamine, serotonin) and the secondary mediators which are synthesized following cell activation (e.g. products of arachidonic acid metabolism such as the leukotrienes). Some of the biochemical steps described include

(1) receptor cross-linking, (2) proteolytic-enzyme activation, (3) changes in membrane phospholipids with the hydrolysis of inositol phosphatides due to phospholipase C, (4) increased intracellular cyclic AMP levels, (5) protein phosphorylation (6) Ca^{2+} influx (7) phospholipase A-2 activation with the release of arachidonic acid and finally the (8) secretion of histamine. A series of variants of the rat basophilic leukemia cells have also been selected which do not release histamine. The experiments during this past year have continued the study of variants of the rat basophilic leukemia cell line and also the biochemical changes which occur during the release of mediators.

We have previously isolated 3 monoclonal antibodies (mAb) that bind to the high affinity IgE receptor on the rat basophilic leukemia cells. One of these mAb (mAb BC4) was purified and coupled to Sepharose 4B beads. Supernatant from extracts of rat basophilic leukemia cells were bound to these affinity beads; after extensive washing in buffer containing detergent the receptors were eluted. Silver staining of the eluate separated by SDS-PAGE demonstrated the 3 receptor components: the broad band at 46-65 kDa; narrow doublet at 30-33 kDa and a broad band at 20 kDa. There were also two other bands at 96 kDa and 43-45 kDa. On immunoblotting with this purified material, ^{125}I-IgE reacted with the broad 46-65 kDa bands and 2 narrow bands at 43-45. On immunoblots with the mAb BC4, there was some reaction with the broad band at 46-65 kDa. The receptor proteins have also been further purified into individual components. The peptides have then been used for amino acid sequence analysis. The N-terminal or the α-chain was blocked; therefore the purified protein was digested with trypsin and the peptides were separated and sequenced. Using the data from this sequence, oligonucleotide probes were synthesized and used to clone and determine the entire nucleotide sequence of cDNAs corresponding to the putative α subunits of the human and rat mast cell high-affinity IgE receptors. Both human and rat cDNAs encode an NH_2-terminal signal peptide, two immunoglobulin-like extracellular domains (encoded by discrete exons), a hydrophobic transmembrane region, and a positively charged cytoplasmic tail. The human and rat α subunits share an overall homology with one another and the immunoglobulin gene family, suggesting that they arose from a common ancestral gene and continue to share structural homology with their ligands. In addition, the rat gene is transcribed into at least three distinct forms, each of which yields a somewhat different coding sequence.

Several monoclonal antibodies have been isolated that inhibit the binding of IgE to receptors on rat basophilic leukemia cells. One of these antibodies, AA4, inhibits histamine release, but does not block the binding of other anti-IgE receptor antibodies to the cell surfaces. The number of AA4 molecules bound per cell is 14 times the number of IgE receptors and many different glycoproteins react AA4 as determined by Western blot analysis. By immunostaining thin layer chromatograms of lipid extracts from rat basophilic leukemia cell line, RBL-2H3, antibody AA4 binds 2 large closely migrating glycolipids. Both glycolipids are disialogangliosides. Sialic acid is required for binding as treatment with neuraminidase destroys binding in a solid phase radioimmunoassay. Both ganglioside antigens were purified from the disialoganglioside fraction by short bed continuous chromatography on thin layer silica gel plates. The structure of the faster migrating glycolipid was analyzed by fast atom bombardment mass spectrometry, (FABMS). Fragment ions indicated the ceramide acyl moiety to be lignoceryl and the carbohydrate composition to consist of three hexoses, one hexosamine, two sialic acids and one deoxyhexose. The molecular weight was determined to be 2067 daltons. The carbohydrate composition and molecular weight values were corroborated by further FABMS analysis using the peracetyl and permethyl derivatives. The carbohydrate sequence is being analyzed by direct chemical ionization mass spectrometry. Therefore, this mAb binds to surface glycolipids that are associated with the receptor and blocks the binding of IgE.

The effect of monoclonal antibody AA4 on the RBL-2H3 cells is also under investigation. As described above, AA4 binds to a glycolipid on the cell surface of the RBL-2H3 cells which is associated with, but not part of the Fc_ϵ receptor. When AA4 is bound to the cell surface, as long as it is not cross linked, it remains evenly distributed on the surface. If AA4 is cross linked, it rapidly caps. Immediately after binding AA4, the RBL-2H3 cells undergo striking morphological changes which appear identical to the changes seen when the Fc_ϵ receptor is activated. However, the changes produced by AA4 are not accompanied by histamine release. When the cells are exposed to AA4, the surface begins to ruffle, and with time, the cells lose their normal spindle shaped appearance and begin to spread. These changes are accompanied by a polymerization and redistribution of actin within the cells, as determined by staining with FITC-phalloidin. There is an increase in staining of actin filaments adjacent to the plasma membrane as well as increased staining within the cell body. Using a mAb against tubulin, AA4 also results in differences in the distribution of microtubules. None of the changes associated with AA4 binding are seen when the cells are exposed to either nonspecific IgG or IgE, nor are they affected by binding AA4 at 4°C or in the absence of Ca^{++}. Recent studies have suggested that AA4 is eliciting these changes by activating protein kinase C. Inhibitors of protein kinase C also inhibit the changes produced by binding AA4. When mutant RBL-2H3 cells which lack protein kinase C 1 and 2 are exposed to AA4 they also fail to respond to the antibody. Furthermore, exposure to the phorbol ester, TPA, an activator of protein kinase C produces the same morphological changes as does AA4. Experiments are in progress to further characterize the relationship with protein kinase C and to determine the biochemical basis for the action of the AA4.

Cross-linked IgE-receptor complexes co-localize with the AA4 antigen on RBL cells. IgE-receptor complexes on rat basophilic leukemia (RBL) cells trigger cellular degranulation when cross-linked by multivalent antigen or specific antibodies. These clusters rapidly associate with the cytoskeletal matrix, suggesting that this may be an important part of the signaling mechanism. We have found that the AA4 antigen is largely detergent-insoluble in a cytoskeleton-stabilizing buffer, remaining associated with the cytoskeletal matrix under conditions that solubilize monomeric IgE-receptor complexes. RBL cells with bound fluorescein-labeled IgE, and AA4 labeled with rhodamine secondary antibody show a uniform distribution of IgE on the cell surface and patches of AA4 that have been clustered by the secondary antibody. When multivalent antigen is also incubated with the RBL cells to cross-link the IgE the resulting IgE patches and the AA4 patches coincide on the cell surface. Fluorescence resonance energy transfer experiments with coumarin-labeled IgE as donor and fluorescein-labeled AA4 as acceptor show that energy transfer occurs between the two probes when the IgE is cross-linked with multivalent antigen. These results indicate that the AA4 antigens become associated with cross-linked IgE receptor complexes, and these antigens may be involved in the transmembrane signal by mediating the receptor-cytoskeletal interaction.

Several components of the high affinity receptor for immunoglobulin E in the rat basophilic leukemia cells are known to be phosphorylated. We investigated the association of a kinase with the subunits of the high affinity receptor. The IgE-receptor was purified by using the anti-receptor mAb BC4 coupled to Sepharose beads. Solubilized rat basophilic leukemia cells were added to the mAb BC4 affinity beads, then washed with buffer before elution with acetic acid. The eluates contained the known receptor components. At different stages in the purification the preparations were tested for endogenous phosphorylation by incubation with [P^{-32}]ATP. There was marked phosphorylation of the beta chain (MW 33 kDa) in the samples that had been washed 3 times and this activity was characteristic of a tyrosine kinase. The activity was decreased after washing beads 6 times and was not detected in the acetic acid eluate containing the receptor components. Therefore, the receptor itself did not have kinase activity, but a tyrosine kinase appears to associate with the receptor during some of the purification steps.

Basophils can be desensitized for IgE-mediated histamine release by challenge under non-optimal conditions. We investigated the capacity of monoclonal antibodies to the high affinity IgE receptor to desensitize cells for histamine release. Fab fragments of IgG monoclonal anti-receptor antibodies did not trigger histamine release from rat basophilic leukemia cells. However, there was release when the cell-bound Fab were crosslinked with anti-mouse Ig antibodies. In contrast, the prolonged incubation of the cells with the Fab anti-receptor antibodies in culture medium at 37°C resulted in the desensitization of the cells for histamine release when challenged with anti-Ig antibodies. This was not accompanied by a change in the amount of ^{125}I-labeled Fab present on the cell surface. For antigen non-specific desensitization, cells were incubated with IgE-anti-TNP and varying amounts of anti-receptor Fab. There was desensitization for antigen-induced histamine release. It has been previously reported that the signal for desensitization required IgE dimers. However, unlike IgE, anti-receptor antibodies can bind to the receptor without crosslinking and activate both a specific and non-specific desensitizing signal.

The secretory response of the parental RBL-2H3 cells usually requires an increase in the intracellular level of Ca^{2+} and activation of protein kinase C (PKC) based on evidence including the synergism between the Ca^{2+} ionophore and tumor-promoting phorbol ester, and the inhibition of secretion by inhibitors of PKC. Several RBL-2H3 cell variants having reduced secretory response to antigen have been established. The level of PKC isozymes in these cells was determined by immunoblot analysis with PKC isozyme-specific antibodies and by chromatographic separation of PKC isozymes on hydroxyapatite column. Among ten 2H3 cell variants tested, all of them contained high levels of the type III PKC. Several mutants having reduced response to antigen for histamine secretion also contained reduced level of the type II PKC. Analysis of the PKC substrates in 2H3 and a type II PKC-deficient mutant cells by in vitro phosphorylation with a proteolytically activated PKC revealed that these two cell lines had the same substrates for PKC. Hence, the different physiological responses observed in the mutant cell line may result from the deficiency of the type II pKC. These findings suggest that the type II PKC is involved in the ligand-mediated secretory response in 2H3 cells.

There are major structural differences between the high affinity and low affinity human IgE receptors. A panel of monoclonal anti-IgE antibodies specific for the F_c -fragment were used to inhibit binding of radio-labeled human IgE(PS) to either the low affinity receptor on B-lymphocytes (RPMI 8866 cell line) or to the high affinity receptor on human basophils. Two monoclonals (mAb 10 and 13) inhibited the binding of IgE to both enriched basophils and the RPMI 8866 cells; two other monoclonals (mAb 11 and 15) inhibited binding only to the basophils. There were two monoclonals (mAb 9 and 14) that inhibited binding only to the RPMI 8866 cells. All these monoclonals recognize distinct epitopes in the C_ϵ 2,3,4 regions of the IgE molecule. Therefore, the sites on IgE that are involved in binding to the receptors on basophils and lymphocytes are different.

Secretory and endocytic process in several cell types are currently under investigation using short term cultures of isolated exocrine acinar cells, a pancreatic acinar cell line (AR42J) and a rat basophilic leukemia cell line (RBL-2H3). Emphasis is placed on morphological, cytochemical and biochemical characterization of these processes in the cultured cells. Events involved in both fluid phase and receptor mediated endocytosis are under investigation. The lysosomal system and its role in endocytic and secretory pathways is also under study. In the past year emphasis has been placed on defining the endocytic pathway of ligands to the Fc_ϵ receptor in RBL-2H3 cells, in characterizing the morphological effects of a monoclonal antibody (AA4) to the surface of the RBL-

2H3 cells, and to defining the lysosomal system in the AR42J cells.

The internalization of ligands to the Fc_ϵ receptor has been followed both by electron microscopy and by density gradient centrifugation. For electron microscopy, a monocolonal antibody to the Fc_ϵ receptor (BC4) and antigen for IgE (DNP-HSA) were each conjugated to colloidal gold. The rat basophilic leukemia cells were exposed to the probes, and fixed for electron microscopic examination at various time intervals. Some cells were also allowed to endocytoze transferrin conjugated to horseradish peroxidase (HRP) in order to label early endocytic compartments. Selected samples were reacted for acid phosphatase to identify lysosomes. Both probes behaved in essentially the same manner. They were rapidly internalized from the cell surface in coated pits and vesicles. By 5 minutes the tracers and the transferrin were co-localized in early endosomes. At approximately 30 minutes of uptake gold particles could be identified in late endosomal compartments (CURL). Both the BC4- gold and the DNP-HSA-gold took about one hour to traverse the early and late endosomal compartments before reaching lysosomes. After 1-2 hours exposure to the tracers, essentially all of it was localized in lysosomes. The transit time from cell surface to lysosomes is somewhat longer than seen for many other cell types where ligand initially reaches the lysosomes by 30 minutes. In order to verify these results, and to examine the fate of IgE itself, ^{125}I labeled BC4 and IgE were used in combination with cell fractionation and density gradient centrifugation to study the endocytic pathways. The results from these studies confirmed the data gathered by electron microscopy. The major difference seen between the IgE and the BC4 was that the amount ^{125}I BC4 present in the lysosomal fraction increased with time, while that of the ^{125}I IgE reached a plateau at about 90 minutes. While these experiments provide information on the fate of internalized ligands to the Fc_ϵ receptor, they do not yield data on the fate of the receptor itself. Experiments are now in progress to determine if the ligands are still associated with the receptor within the lysosomes.

The lysosomal system and secretory apparatus in the AR42J cells has been the focus of other investigations. In vivo, exocrine acinar cells contain two populations of lysosomes. One population is present in the Golgi region and contains both acid phosphatase (AcPase) and trimetaphosphatase (TMPase), while a second population of basally located tubular lysosomes contains only TMPase. The electron microscopic cytochemical distribution of TMPase in AR42J cells was examined. The AR42J cells can be stimulated to differentiate in vitro. In undifferentiated cells, TMPase activity was primarily restricted to Golgi associated lysosomes and a few short tubular segments along the cis Golgi. In differentiated cells, TMPase was localized in AcPase positive secondary lysosomes near the Golgi complex and also in AcPase negative lysosomes in peripheral regions of the cells. In addition, TMPase positive tubules and vesicles that were equivalent to the basal tubular lysosomes of exocrine cells in vivo were found peripherally in the cells and in some instances extended for up to 1 μm into the cells. A second striking localization of the TMPase was in a saccule present along the cis Golgi which, when viewed en face, had a fenestrated appearance. Some mature secretory granules also contained TMPase activity around their peripheries. The distribution of TMPase was then compared to that of AcPase, which in exocrine acinar cells *in vivo* is predominately associated with the secretory apparatus. AcPase activity in the AR42J cells was localized predominately in the Golgi region where it was found in the trans-most Golgi saccules (GERL), and in immature secretory granules. AcPase was also localized in large spherical lysosomes adjacent to the Golgi apparatus. In contrast, TMPase activity could be demonstrated in lysosomes throughout the cell, as described above. The cells were also reacted with an antibody (A gift from Dr. Ira Mellman, Yale University) against a lysosomal membrane glycoprotein (GP 120). The antibody reacted with both the spherical lysosomes adjacent to the Golgi apparatus and the tubular peripheral lysosomes but not with the AcPase positive saccules at the trans face of the Golgi apparatus or with the TMPase positive cis Golgi saccules. These results show that differentiated AR42J cells do possess two morphologically and cytochemically distinct populations of lysosomes similar to those seen *in vivo*. This cell line should prove useful in elucidating the function of lysosomes in exocrine acinar cells. Studies are in progress to assess the function of these lysosomes *in vitro*. Initial efforts have concentrated on the uptake of HRP and transferrin-HRP into these cells. While soluble HRP is localized in lysosomes after approximately 30 minutes uptake, the transferrin, still bound to its receptor, is recycled to the cell surface and intracellularly remains in endocytic compartments. The intracellular fate of other membrane bound ligands and their relationship to the lysosomal system is currently under investigation. The role a acid phosphatase in the exocrine acinar cells is also being examined. Monoclonal antibodies have been raised against partially purified acid phosphatase from rat parotid acinar cells. Two of the monoclonal antibodies will remove AcPase activity from tissue homogenate in enzyme removal assays. Methods are being developed to transfer enzymatically active AcPase to nitrocellulose paper in order to compare the Western blot of the antibody with the enzymatic activity. The antibodies are also being characterized by standard immunological methods. Since the exocrine acinar cells contain two populations of lysosomes, one AcPase positive, and one AcPase negative, studies on the distribution of AcPase in these cells may provide information on the *in vivo* function of this enzyme.

PUBLICATIONS

Agelli M, Wahl SM. Synthesis of biologically active fibroblast activating factor (FAF) by *Xenopus* oocytes injected with T lymphocyte mRNA. Cell Immunol 1987;110:183-190.

Agelli M, Wahl SM. Collagen production by fibroblasts. Methods in Enzymology. 1988;163:642-656.

Allen JB, Bansal G, Ink L, Hunt D, Hand A, Wahl L, and Wahl S. Recombinant rat gamma interferon (IFN) suppresses streptococcal cell wall induced arthritis in rats. Arthritis Rheum 1988;31(4):S28.

Allen JB, Poy M, Wong J, Wahl L, Wahl S. Activation of mononuclear phagocytes by bacterial peptidoglycans: possible role in the pathogenesis of arthritis. J Leukocyte Biol 1988;In press.

Bickel M, Amstad P, Tsuda H, Sulis C, Asofsky R, Mergenhagen SE, Pluznik DH. Induction of Granulocyte-Macrophage Colony Stimulating Factor by Lipopolysaccharide and anti-Immunoglobulin M Stimulated Murine B cell lines. J Immunol 1987;139:2984-2988.

Bickel M, Wahl SM, Mergenhagen SE, and Pluznik DH. Granulocyte-macrophage colony stimulating factor regulation in murine T cells and its relation to cyclosporin A. Exp Hematol 1988;In press.

Falk LA, Wahl LM, Vogel SN. Analysis of Ia antigen expression in macrophages derived from bone marrow cells cultured in granulocyte-macrophage colony-stimulating factor or macrophage colony-stimulating factor. J Immunol 1988;140:2652-2660.

Francois DT, Katona IM, June CH, Wahl LM, Feuerstein N, Huang K-P, Mond JJ. Anti-Ig-Mediated proliferation of human B cells in the absence of protein kinase C. J Immunol 1988;140:3338-3343.

Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LM, Lane HC, Fauci AS, Burke DS, Skillman D Meltzer MS. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J Exp Med 1988;167:1428-1441.

Heikkila R, Schwab G, Wickstrom E, Loke SL, Pluznik DH, Watt R, Neckers LM. The role of *c-myc* in lymphocyte proliferation: Inhibition of S phase entry but not G_0 to G_1 traversal by a *c-myc* antisense oligodeozynucleotide. Nature 1987;328:445-449.

Janoff EN, Smith PD. Immunology of Giardia infections. *In* Giardiasis. Meyer, E. A. (ed.) Elsevier Science Publishers, Amsterdam. 1988;In press.

Janoff EN, Smith PD. Perspectives on gastrointestinal infections in AIDS. Gastroenterol Clin N Am 1988;In press.

Janoff EN, Smith PD, Blaser M. Antibody responses to *Giardia lamblia* in patients with the acquired immunodeficiency syndrome. J Inf Diseases 1988;157:798-804.

Karten MJ, Hook WA, Siraganian RP, Coy DH, Folkers K, Rivier JE, and Roeske RW. *In vitro* histamine release with LHRH analogs. *In* LHRH and its analogs: contraceptive and therapeutic applications, II. Vickery, B. and Nestor, J. (eds.) MTP Press Ltd., Lancaster, England, 1987.

Kitani S, Kraft D, Fischler C, Mergenhagen SE, Siraganian RP. Inhibition of allergic reactions with monoclonal antibody to the high affinity IgE receptor. J Immunol 1988;140:2585-2588.

Livne E, Oliver C, Silberman M. Further Characterization of the Chondroprogenitor Zone in Mandibular Condyles of Suckling Mice. Acta Anat 1987;129:231-237.

Ljungqvist A, Feng D, Tang P, Kubota M, Okamoto T, Zhang Y, Bowers C, Hook W, Folkers K. Design, synthesis and bioassays of antagonists of LHRH which have high antiovulatory activity and release negligible histamine. Biochem and Biophs Res Commun 1987;148:849-856.

Mergenhagen SE, Sandberg AL, Chassy BM, Brennan MJ, Yeung MK, Donkersloot JA, Cisar JO. Molecular basis of bacterial adhesion in the oral cavity. *In* Perspectives on Microbial Pathogenesis and Host Defense. Urbaschek, B. (ed.) U. of Chicago Press, 1988, In press.

Metzger Z, Hoffeld JT, Charon J, Mergenhagen SE. Suppression of lymphocyte and fibroblast proliferation by *Actinomyces viscosus* - activated murine macrophages. J Periodontol Res 1987;22:456-460.

Neckers LM, Tsuda H, Weiss E, Pluznik DH. Differential expression of *c-myc* and transferrin receptor in G_1 synchronized M1 myeloid leukemia cells. J Cell Physiol 1988;135:339-344.

Oliver C. Carboxylic Ester Hydrolases, Lipase, Aryulsulphatase and Carbonic Anhydrase. *In* Histochemistry. Pearse, A. G. E. and Stoward, P. (eds.) London, Chapman Hall, 1988, In Press.

Oliver C, Tolbert CL, Waters JF. Internalization of Horseradish peroxidase isozymes by pancreatic acinar cells *in vitro*. J Histochem Cytochem 1988;In Press.

Oliver C, Waters JF, Tolbert CL, Kleinman HK. Growth of exocrine acinar cells on a reconstituted basement membrane gel. In Vitro Cell Devel Biol 1987;23:465-473.

Prosser FH, Wahl LM. Involvement of the ornithine decarboxylase pathway in macrophage collagenase production. Arch Biochem Biophys 1988;260:218-225.

Pluznik DH, Bickel M, Mergenhagen SE. B lymphocyte-derived Hematopoietic growth factors. J Immunol Investigations 1988;In press.

Shimizu A, Tepler I, Benfey P, Berenstein E, Siraganian R, Leder P. Human and rat mast cell high-affinity immunoglobulin E receptors: characterization of putative α-chain products. Proc Natl Acad Sci 1988;85:1907-1911.

Singh VK, Maheshwari RK, Damewood GP, IV, Oliver C, Friedman RM. Interferon Alters Intracellular Transport of Vesicular Stomatitis Virus Glycoprotein. J Biol Reg Homeostat 1988;In Press.

Siraganian RP. Allergy to animals - principles of clinical management. NER Allergy Proc 1987;8:18-20.

Siraganian RP. The cell biology of mast cells and basophils. In Inflammation: Basic Principles and Clinical Correlates. Gallin J, Goldstein I, Snyderman R (eds.) Raven Press Ltd., New York, 1988.

Smith PD. Giardia lamblia. In Parasitic Diseases in the Immunocompromised Host. Genta RM, Walzer PD (eds.) Plenum Publishing Co., New York, 1988, 343-384.

Smith, PD. Infections of the gastrointestinal tract in the acquired immunodeficiency syndrome. In Mucosal Immunity and Infections on Mucosal Surfaces. Strober W, James S, Lamm ME McGhee JR (eds.) Oxford University Press, New York, 1988, 490-499.

Smith PD. Infections in the immunosuppressed host. In The Large Intestine: Physiology, Pathophysiology and Diseases. Philips SF, Pemberton JH, Shorter RG (eds.) Raven Press, New York, NY, 1988, In press.

Smith PD. Parasitic infections in the gastrointestinal tract. In Immunology and immunopathology of the Liver and Gastrointestinal Tract. Targan SR, Sahnahan F (eds.) Igaku-Shoin Medical Publishers Inc, 1988, In press.

Smith PD, Janoff EN. Infectious diarrhea in human immunodeficiency virus infection. Gastroenterol Clin N Am In press.

Smith PD, Lane HC, Gill VJ, Manischewitz J, Quinnan GV, Fauci AS, Masur H. Intestinal infections in the acquired immunodeficiency syndrome: etiology and therapy. Ann Intern Med 1988;108:328-333.

Smith PD, Lamerson, CL, Wahl LM, Wahl SM. Granulocyte-macrophage colony-stimulating factor enhances effector and accessory cell function by circulating human momocytes. J Cell Biochem 1988;In press.

Smith PD, Wahl SM. Cytokines in the immune response. In Natural Immunity. Nelson DS (ed.) Harcourt Brac Jovanovich Group, North Rycle, Australia, 1988, In press.

Smith PD, Wahl SM. Modulation of fiber formation and possible therapeutic application. Testa, B. and Perrissoud, D. (eds.). CRC Press Inc., Boca Raton, FL, 1988, 223-232.

Wahl SM. Hepatic granuloma as a model of inflammation and repair: an overview. Methods in Enzymology. 1988;163: 605-622.

Wahl SM. Lymphocyte and macrophage-derived growth factors. Methods in Enzymology 1988;163:715-731.

Wahl SM. Fibrosis: Bacterial cell wall induced hepatic granulomas. In Inflammation: Basic Principles and Clinical Correlates. Gallin JI, Goldstein I, Snyderman R (eds.) Raven Press, Ltd., New York, 1988, 841-860.

Wahl SM. Corticosteroids and wound healing. In Antiinflammatory steroid action: basic and clinical aspects. Schleimer RP (ed.) Academic press, 1988, In press.

Wahl SM, Allen JB. T lymphocyte dependent mechanisms of fibrosis. In Growth Factors and Other Aspects of Wound Healing: Biological and Clinical Implications. Alan R. Liss, Inc. 1988, 147-160.

Wahl SM, Hunt DA, Wakefield L, McCartney-Francis N, Wahl L, Roberts A, Sporn MB. Transforming growth factor beta (TGF-ß) induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci 1987;84:5788-5792.

Wahl SM, McCartney-Francis N, Hunt DA, Smith PD, Wahl LM, Katona IM. Monocyte interleukin 2 receptor gene expression and interleukin 2 augmentation of microbicidal activity. J Immunol 1987;139:1342-1347.

Wahl SM. Acute and chronic inflammation. In Human Monocytes. Asherson GL, Zembala M (eds.) Academic Press 1988;In press.

Wahl SM. Cellular interactions in chronic inflammation. In Cell Mediated Immunity in Relation to Tropical Diseases. 1988;In press.

Wahl SM. Monocytes and granulomatous inflammatory responses. Cytokines. 1988;In press.

Wahl SM, Hunt DA, Bansal G, McCartney-Francis N, Ellingsworth L, Allen JB. Bacterial cell wall induced immunosuppression: Role of transforming growth factor beta. J Exp Med 1988;In press.

Wahl SM, Hunt DA, Wong HL, Dougherty S, McCartney-Francis N, Wahl LM, Ellingsworth L, Schmidt JA, Hall G, Roberts GB, Sporn, MB. Transforming growth factor beta is a potent immunosuppressive agent which inhibits interleukin-1-dependent lymphocyte proliferation. J Immunol 1988;140:3026-3032.

Wahl SM, Wahl LM. Inflammation. In Wound Healing: Biochemical and Clinical Aspects. Cohen, IK, Diegelman RF, Lindblad WJ (eds.) 1988;In press.

Wahl SM, Wong HL, McCartney-Francis, N. Growth factors from immune cells. J Cell Biochem 1988;In press.

Waldman, BC, Oliver C, Krag SS. A Clonal Derivative of Tunicamycin-Resistant Chinese Hamster Ovary Cells with Increased N-Acetylglucosamine-Phosphate Transferase Activity has Altered Asparagine-Linked Glycosylation. J Cell Physiol 1987;131:302-317.

Whitcomb RW, Linehan WM, Wahl LM, Knazek RA. Monocytes stimulate cortisol production by cultured human adrenocortical cells. J Clin Endocrinol Metab 1988;66:33-38.

Wientroub S, Winter CC, Wahl SM, Wahl LM. Effect of vitamin D deficiency on macrophage and lymphocyte function in the rat. Calcif Tissue Int 1988;In press.

WoldeMusie E, Ali H, Takaishi T, Siraganian RP, Beaven M. Identification of variants of the basophilic leukemia (RBL-2H3) cells that have defective phosphoinositide responses to antigen and stimulants of GTP-regulatory proteins. J Immunol 1987;139:2431-2438.

Wong HL, Wahl SM. Inflammation and Repair. In Peptide Growth Factors. Handbook of Experimental Pharmacology. 1988;In press.

Wong HL, Wahl SM. Tissue repair and fibrosis. In Human Monocytes. Asherson GL, Zembala M (eds.) Academic Press. 1988;In press.

Yocum DE, Klippel JH, Wilder RL, Gerber NL, Austin HA, Wahl SM, Lesko L, Minor JR, Preuss HG, Yarboro C, Berkebile C Dougherty S. Cyclosporin A in severe, treatmentrefractory rheumatoid arthritis: A randomized study. Ann Intern Med 1988;In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00034-20 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Histmaine Release

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	LMI NIDR
Hook, William A.	Research Microbiologist	LMI NIDR
Berenstein, Elsa H.	Microbiologist	LMI NIDR
Kitani, Seiichi	Visiting Fellow	LMI NIDR
Teshima, Reiko	Visiting Fellow	LMI NIDR
Bishop, Brian	Biologist	LMI NIDR

COOPERATING UNITS *(if any)*

NHLBI, Laboratory of Chemical Pharmacology, NIH (M. Beaven:) NICHD, ODCPR, NIH (M. Karten).

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	1.5	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, the anaphylatoxins, LHRH peptides, and the Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00046-17 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic inflammation and immunomodulation of connective tissue metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Wahl, Sharon	Chief, Cellular Immunology Section	LMI	NIDR
Allen, Janice	Chemist	LMI	NIDR
Hunt, Denise	Microbiologist	LMI	NIDR
McCartney-Francis, Nancy	Microbiologist	LMI	NIDR
Dougherty, Suanne	Microbiologist	LMI	NIDR
Wong, Henry	Staff Fellow	LMI	NIDR
Welch, Glenn	Biologist	LMI	NIDR

COOPERATING UNITS (if any)

I. Katona, USUHS; M. Sporn, A. Roberts, LC, NCI; L. Ellingsworth, Collagen Corporation.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.30	1.70	4.60

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mononuclear cells generate a variety of hormone-like proteins termed growth factors which are instrumental in the evolution and resolution of inflammatory reactions. Many of these growth-regulatory molecules have multi-functional properties. For example, transforming growth factor beta (TGF-β) is a potent chemoattractant and functions as an inducing agent for other monocyte-derived growth hormones. Once secreted, these growth-promoting molecules provide a network of signals which regulate other inflammatory cells as well as fibroblast proliferation and angiogenesis. Although TGF-β induces IL1 production, it is also a potent inhibitor of IL1-dependent lymphocyte proliferation. Inhibition of cell division occurs late in the activation sequence of the lymphocytes since neither gene expression of IL2 or IL2 receptors is suppressed. Our recent studies have documented enhanced levels of TGF-β within a chronic inflammatory lesion characterized by profound immunosuppression. This immunosuppressive activity was blocked with an antibody to TGF-β. The primary source of this TGF-β within the lesion appears to be the macrophages. Macrophages constitutively express TGF-β mRNA but only synthesize and secrete the polypeptide when stimulated. These observations suggest that TGF-β might provide a negative feedback mechanism to reverse the inflammatory response, yet enable IL1, TNF and other monokines to continue modulation of tissue repair. Thus, the biological effects of TGF-β may serve to protect the host from prolonged inflammatory events and to promote the healing of inflamed tissues.

In addition to the secretion of growth hormones, monocytes activated at an inflammatory site lose their ability to chemotax to certain inflammatory stimuli. One mechanism for this cessation of migration appears to be the modulation of chemotactic ligand receptors. Activation by bacterial products, certain viruses and/or lymphocyte products (γIFN) causes a down-regulation of C5a receptors and a corresponding loss of ability to chemotax to C5a. Consequently, monocytes, once activated, likely become unable to migrate further to inflammatory stimuli and therefore accumulate at the inflammatory site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00199-12 LMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Oliver, Constance	Research Biologist	LMI NIDR
Others:	Hart, Tim	IRTA	LMI NIDR
	Sahara, Noriyuki	Visiting Fellow	LMI NIDR
	Tolbert, Carolyn L.	Microbiologist	LMI NIDR
	Waters, Judith F.	Biologist	LMI NIDR

COOPERATING UNITS *(if any)*

Dr. A. Robbins, GB, NIDDK

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.75	1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Secretory and endocytic process in several cell types are currently under investigation. Short term cultures of isolated exocrine acinar cells, a pancreatic acinar cell line (AR42J), a rat basophilic leukemia cell line (RBL-2H3), and other cultured cells are being used to study various aspects of endocytic and secretory processes. Emphasis is placed on morphological, cytochemical and biochemical characterization of these processes in the cultured cells. Events involved in both fluid phase and receptor mediated endocytosis are under investigation. The lysosomal system and its role in endocytic and secretory pathways is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00254-11 LMI

PERIOD COVERED
October 1, 1987 - September 1, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cisar, John O.	Microbiologist	LMI	NIDR
Yeung, Maria K.	Post-Doctoral Fellow	LMI	NIDR
Hsu, S. Dana	Microbiologist	LMI	NIDR
Sandberg, Ann L.	Chief, Humoral Immunity Section	LMI	NIDR

COOPERATING UNITS *(if any)*
W.B. Clark, University of Florida; F.C. McIntire and A.E. Vatter, University of Colorado; C.A. Bush, Ill. Inst. Technology; Dr. M. Kilian, Aarhus, Denmark

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Humoral Immunity Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.0	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The subunits of Actinomyces viscosus T14V type 1 and Actinomyces naeslundii WVU45 type 2 fimbriae have been identified by cloning and expression of the respective genes in Escherichia coli. The amino acid sequence of each subunit was deduced from the nucleotide sequence of the cloned gene and the N-terminal sequence confirmed by Edman degradation of purified fimbriae and cloned fimbrial subunit. The genes for type 1 and type 2 fimbrial subunits encode proteins of approximately 530 amino acid residues of which the first 30 to 32 appear to be signal sequences. An evolutionary relationship between the subunits of Actinomyces spp. type 1 and type 2 fimbriae was evident from amino acid sequence homologies. Studies of bacterial adherence using A. viscosus T14V and mutants specifically lacking type 1, type 2 or both types of fimbriae have provided evidence that the acidic proline rich proteins are specific receptors for type 1 fimbriae. These salivary components are not glycosylated and thus, their recognition by A. viscosus type 1 fimbriae most likely depends on protein-protein interactions. In contrast, the receptor molecule on Streptococcus sanguis 34 for Actinomyces spp. type 2 fimbriae is a linear polysaccharide composed of phosphodiester-linked hexasaccharide subunits. The reaction of rabbit antibody with this polysaccharide depends on the nonreducing end of the hexasaccharide chain while GalNAcβ1→3Gal at the reducing end is involved in lectin recognition. A similar separation of the antigenic determinant and receptor structure occurs within the polysaccharide of S. sanguis J22. However, with strain J22, Galβ1→3GalNAc at the reducing end of the oligosaccharide subunit appears to be involved in lectin recognition. Thus, the receptor activity of these streptococcal polysaccharides may depend on internal structural features that mimic the glycoprotein and glycolipid receptors on host cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE-00290-09 LMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Production of Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	LMI NIDR
Hook, William A.	Research Microbiologist	LMI NIDR
Berenstein, Elsa H.	Microbiologist	LMI NIDR
Fischler, Cynthia	Bio Lab Tech Microbiol	LMI NIDR
Zinsser, Frank U.	Guest Worker	LMI NIDR
Mergenhagen, Stephan E.	Chief, LMI	LMI NIDR
Kitani, Seiichi	Visiting Fellow	LMI NIDR
Roberts, Barbara A.	Biologist	LMI NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	1.9	2.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the FCε receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00374-06 LMI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Action and regulation of colony stimulating factors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

Dov H. Pluznik	Expert	LMI	NIDR
Matthias Bickel	Visiting Associate	LMI	NIDR
Lynda L. Weedon	Biologist	LMI	NIDR
Stephan E. Mergenhagen	Chief, LMI	LMI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.00	2.90	1.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present report describes our results on: a) Molecular control of GM-CSF production and b) Synergism between CSF and IL-1 in inducing differentiation in myeloid cells. a) Based on previous experiments we hypothesized that the production of GM-CSF in T cells is under post-transcriptional control. The present report describes our experimental results in supporting this hypothesis. Northern analysis was used to determine the steady-state levels of GM-CSF mRNA and nuclear transcription analysis (runoff) was performed to determine transcriptional activity. GM-CSF mRNA could be detected only in TPA-stimulated EL-4 thymoma cells but not in unstimulated cells. However, treatment of unstimulated cells with cyclohexemide enabled detection of GM-CSF mRNA by Northern analysis. Furthermore, protecting GM-CSF RNA transcripts isolated from unstimulated cells with complementary ^{32}P-labeled RNA and separation of the hybrids on CF11 cellulose columns revealed the presence of GM-CSF mRNA. Additonal experiments showed transcriptional activity of the GM-CSF gene in unstimulated cells. Moreover, utilizing a drug that inhibits transcription initiation had no effect on production of GM-CSF in stimulated cells. All these data support a post-transcriptional control of GM-CSF production. b) CSF present in mouse lung conditioned medium (MLCM) induces differentiation in the myeloid leukemia cell line, M1, to mature macrophages. Optimal concentraions of MLCM (10%) induce a sequential expression of differentiation markers such as FcR, C3bR, induction of lysozyme and morphological changes typical of terminal myeloid differentiation. Suboptimal concentrations of MLCM (1.25-2.5%) fail to induce full differentiation. However, when IL-1α is added to such suboptimal concentrations of MLCM, the combination of both cytokines is followed by a complete sequence of differentiation similar to that of optimal concentrations of MLCM. These data support the suggestion that IL-1α has an effect on hematopoietic cells, and is capable of cooperating with CSF in inducing differentaition in myeloid cells.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00392-05 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monocyte/Macrophage Function in the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Phillip D. Smith, M.D.	Senior Staff Fellow	LMI	NIDR
Sharon M. Wahl, Ph.D.	Chief, Cellular Immunology Section	LMI	NIDR
Larry M. Wahl, Ph.D.	Microbiologist	LMI	NIDR
Cindy L. Lamerson	Biologist	LMI	NIDR
William L. Whelan, Ph.D.	Visiting Associate	LMI	NIDR
Edward N. Janoff, M.D.	Visiting Investigator	LMI	NIDR
Romi Saini	Medical Student	LMI	NIDR

COOPERATING UNITS *(if any)* Dr. J.M. Orenstein, GW University Medical Center; Dr. M. Popovic, NCI; Dr. S. Gartner, NCI; Dr. R. Yarchoan, NCI; Dr. S. Broder, NCI; Dr. A. Suffridini, NIAID; Dr. H. Masur, NIAID; Dr. M.S. Chernick, NIDDK

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.40	2.60	1.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunosuppression in patients with AIDS is often out of proportion to the level of CD4+ lymphocyte depletion. Moreover, for many of the infections in AIDS patients the monocyte-macrophage is the primary host defense cell. These observations, together with recent evidence that monocytes also are a target of HIV infection, suggest the possibility that the virus itself may induce defective monocyte function. Therefore, the focus of this laboratory has been monocyte dysfunction in AIDS. Our results indicate that HIV alters spontaneous and stimulated production of 0-2 a key reactive oxygen intermediate involved in oxygen-dependent cytotoxicity and tissue inflammatory changes. Preliminary results also indicate that uptake of HIV by mucosal M cells may be a mechanism of viral entry into the host. Relevant to vaccine development, we have found that IgA, but not IgG, antibodies to HIV are absent in the intestine. Experiments to determine whether HIV infection of monocytes alters B cell Ig secretion are in progress. We have shown that cytomegalovirus, the most common opportunistic infection in AIDS patients, impairs accessory cell function and stimulates tumor necrosis factor secretion. Our observations on the ability of GM-CSF to augment 0-2 production, cytotoxicity, and HLA-DR expression either in vitro or in vivo are clinically relevant to the ongoing trials of GM-CSF therapy in AIDS patients. Also, DNA restriction patterns of C. albicans from AIDS patients are similar to isolates from normal subjects; indicating that the immune response, as opposed to a specific genotype, is the critical factor in the development of candidiasis in patients. Therefore, we are investigating the mechanism of monocyte killing of Candida. Studies on the contribution of alveolar macrophages to the pulmonary inflammatory changes observed in AIDS, endotoxic shock, and cystic fibrosis, to compare to the role of the alveolar macrophage in AIDS patients are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00424-03 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine Expression in Immunological Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

McCartney-Francis, Nancy L.	Senior Staff Fellow	LMI	NIDR
Smith, Phillip D.	Senior Staff Fellow	LMI	NIDR
Wahl, Larry M.	Microbiologist	LMI	NIDR
Wahl, Sharon M.	Chief, Cellular Immunology	LMI	NIDR
Mizel, Diane	Chemist	LMI	NIDR
Dougherty, Suanne	Biologist	LMI	NIDR
Wong, Henry	Staff Fellow	LMI	NIDR
Allen, Janice	Chemist	LMI	NIDR

COOPERATING UNITS *(if any)*

Suzanne Gartner, Ph.D., Mikulas Popovic, Ph.D. NCI., NIH, Gary L. Simon, MD., Ph.D.
Gary L. Simon, MD., Ph.D., George Washington University, Michael B. Sporn,
Anita B. Roberts, NCI, NIH.

LAB/BRANCH

Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this research program are to define the cytokines elaborated by the cells involved in the inflammatory process and in various disease conditions and to describe the mechanisms that regulate cytokine expression. Transforming growth factor-beta (TGF-β) induces monocyte chemotaxis and growth factor production, specifically interleukin-1 (IL-1). platelet-derived growth factor (c-sis), tumor necrosis factor-α (TNF-α), and fibroblast growth factor (FGF), yet blocks the ability of IL-1 to stimulate lymphocyte proliferation. In addition, TGF-β upregulates expression of TGF-β mRNA, while lipopolysaccharide (LPS) causes a reduction in the basal level of TGF-β mRNA. This reduction of TGF-β message can be reversed in the presence of cycloheximide, suggesting that a regulatory protein may control the expression of the TGF-β gene. Activated monocytes secrete TGF-β protein and treatment with anti-TGF-β antibodies blocks expression of IL-1 RNA, suggesting that IL-1 production by activated monocytes occurs through the action of newly-released TGF-β. Differential expression of TGF-β may contribute to the outcome of an inflammatory lesion by promoting wound healing while limiting tissue damage. Inappropriate expression of cytokines and cell surface antigens may play an important role in disease conditions. Increased levels of IL-1, TNF-α, and IL-2R mRNAs are seen within 2 to 4 hr after in vitro exposure to HIV-1, suggesting that the virus is acting as an activating agent. Spontaneous and LPS-induced levels of TNF-α, IL-1, TGF-β, and granulocyte-macrophage colony stimulating factor (GM-CSF) RNAs are increased in monocyte cultures where viral replication is rapidly increasing. During this same period, expression of RNAs encoding the cell surface proteins IL-2R, HLA-DR, and T4 is dramatically decreased in HIV-infected monocytes. The modulation of cytokine and cell surface protein expression in HIV-infected monocytes suggests that transactivating elements which regulate HIV expression may also contribute to the activation or inactivation of certain host genes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00441-02 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization and Immunoregulation of Experimentally Induced Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen, Janice Benson	Chemist	LMI	NIDR
Wahl, Sharon	Chief, Cellular Immunology Section	LMI	NIDR
Feldman, Gerald	NRSA Research Associateship	LMI	NIDR
Bansal, Geetha	Guest Worker	LMI	NIDR
Skaleric, Uros	Fogarty Scholar	LMI	NIDR
Wright, Lori	Stay-in-School Student	LMI	NIDR
Wahl, Larry	Microbiologist	LMI	NIDR
Pilcher, Marshall	Biologist	LMI	NIDR

COOPERATING UNITS *(if any)* E. Amento, Genentech; J. Coffey, Hoffman LaRoche; A. Hand, LOP, NIDR; L. Ellingsworth, Collagen Corporation; M. Bolander, LDBA, NIDR; R. Simmons, Syntex; N. Cunningham, MTRB, NIDR

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.15	2.95	2.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Group A streptococcal cell walls (SCW) induce a biphasic pattern of chronic, erosive polyarthritis in the LEW, but not the genetically similar F344, rat. In studies to identify the cellular and molecular mechanisms of differential genetic susceptibility, peritoneal macrophages from the two strains were compared for their responsiveness to SCW in vitro and in vivo. LEW macrophages produced higher levels of differentiation and proliferative factors and reactive oxygen intermediates as compared to the F344, all of which promote chronic inflammation and may provide a basis for the differential development of disease. To determine the contribution of these factors relative to the two phases of arthritis, studies were performed using specific inhibitors. Administration of the cyclooxygenase inhibitors flurbiprofen and ketoprofen markedly suppressed both the acute and chronic phases of arthritis, as well as established arthritis, suggesting that cyclooxygenase metabolites of arachidonic acid contribute to the initiation and maintenance of this response. In contrast, nordihydroguaiaretic acid, a lipoxygenase inhibitor, had no effect on the acute with minor effects on the chronic phase. In additional studies, administration of gamma interferon (γIFN) suppressed both the acute and chronic phases of disease, possibly due to its antiproliferative effects and its inhibitory effects on prostaglandin and collagenase synthesis. These studies reflect the effects of γIFN in human rheumatoid arthritis observed in ongoing clincial trials. By choosing pharmacologic agents with different target specificities, it may be possible to elucidate the mechanisms of pathogenesis of arthritis, in both experimental models and in humans.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE-00456-01 LMI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell activation: relationship to connective tissue and immunodeficiency diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Wahl, Larry M.	Research Biologist	LMI NIDR
Wahl, Sharon M.	Research Microbiologist	LMI NIDR
Corcoran, Marta L.	Chemist	LMI NIDR

COOPERATING UNITS *(if any)* I. Katona, USUHS; S. Vogel, USUHS; D. S. Finbloom, FDA; W. L. Farrar, NCE; L. O. Arthur, NCI; S. W. Pyle, NCI; M. S. Meltzer, Walter Reed and H. E. Gendelman, Walter Reed

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our experiments on the regulation of monocyte collagenase production by recombinant interferon-γ (rIFN-γ) have demonstrated that rIFN-γ inhibited collagenase synthesis as a result of its ability to block PGE2 production. The mechanism of rIFN-γ inhibition of PGE2 synthesis is due to a reduction in phospholipase A2 (PLA2) activity based on: 1) the ability of rIFN-γ to inhibit, as assessed by HPLC, the release of arachidonic acid metabolites and 2) measurement of the PLA2 in the membranes of monocytes demonstrat-ed that rIFN-γ significantly reduced the membrane associated PLA2 activity. In our studies on human immunodeficiency virus type 1 (HIV-1) and the monocyte, we examined the affect of the envelope protein (gp120) on the function of these cells. Addition of purified gp120 to monocytes resulted in the induction of interleukin 1 (IL 1) and arachidonic acid metabolites from the cyclooxygenase and lipoxygenase pathways. Cytofluorometric analysis revealed that binding of OKT4a to the CD4 on monocytes was blocked by gp120, whereas OKT4 was unaffected. These findings demonstrate that gp120 is directly involved in the transduction of a signal, possibly through the OKT4a epitope on the CD4 receptor. Further evidence for CD4 mediated signal transduction was obtained with the use of monoclonals OKT4 and OKT4a which induced PGE2 and IL 1 production. Additionally, gp120 caused a decrease in FMLP and C5a receptors on monocytes which impaired their respond to a chemotactic signal. In contrast, IL 2 receptors on monocytes are increased by gp120. Similar changes are observed after the initial exposure of monocytes to the Ba-L strain of HIV-1 suggesting that gp120 on the intact virion as well as soluble gp120 can dramatically influence monocyte function.

Annual Report of the Laboratory of Microbial Ecology

The newly formed Laboratory of Microbial Ecology presently consists of the microbiology and microbial pathogenesis groups whose missions are to identify and characterize cell-to-cell interactions among oral microorganisms, interactions between oral microorganisms and host tissue, and host responses to the oral flora. Studies on the physiology and genetics of oral and lactic acid bacteria are an essential component of this group's mission. To this end, members of the new laboratory are characterizing interactions (coaggregations) between genetically diverse microorganisms found above and below the gingival margin of the human oral cavity. These coaggregations, many of which are lectin-carbohydrate mediated reactions, are currently being evaluated to determine whether the coaggregation patterns can be related to the ecological niches inhabited by the participating partners and whether complex mixtures of bacteria are capable of accreting in a temporal or sequential fashion. Concomitantly, the molecular nature of the mediators of coaggregation is under investigation. These studies are presently elucidating the composition and structural properties of the adhesins, receptors and associated surface structures that mediate coaggregation and tissue tropisms. Complementary investigations show how the adhesins and receptor molecules are arranged on the surface of the bacterial cell and their relative numbers per cell.

Physiological and genetic studies characterizing the metabolic processes essential for survival of the oral microbiota and regulation of the genetic systems responsible for their synthesis are a necessary element of this group's focus. Investigations center on the transport of saccharides by established and novel mechanisms, the role of amino acid fermentation in hexose transport, the synthesis and function of novel amino acids produced by lactic acid bacteria and the arrangement and regulation of the genes which encode the transport components.

Definition of the molecular basis for recognition of mammalian cell receptors by certain oral microorganisms is providing important insights into the mechanisms involved in colonization of epithelial surfaces and stimulation of phagocytic cells by these bacteria. In addition to identifying, purifying and characterizing the relevant bacterial adhesins, these studies focus on the characterization of their complementary mammalian cell receptors. The biological consequences of the attachment of oral microorganisms to different polymorphonuclear leukocyte receptors are also being examined in detail. This process initiates a sequence of events that can ultimately result in the destruction of the bacteria. However, the concomitant release of reactive oxygen intermediates and granule constituents from these phagocytic cells contributes to inflammation of the surrounding host tissues. Thus, the findings obtained during the course of these investigations have major implications not only in host defense but also in the production of damage to oral tissues.

The Microbiology Section

A vital link has been established between the process of coaggregation and the ecology of the oral cavity. Members of the genus Veillonella are ineffectual colonizers of soft or hard oral tissues, however, they comprise a significant and important part of the oral microflora. Suveying 200 strains of Veillonella, it has recently been demonstrated that the three species of this genus coaggregate with one or more species of oral streptococci or actinomyces. More important yet, two species, V. atypica and V. dispar, are found predominantly on the dorsum of the tongue; both species coaggregate with Streptococcus salivarius a primary colonizer of the tongue. V. parvula is found mainly on hard tissue. It coaggregates with strains of streptococci and actinomyces which are primary colonizers of the tooth surface. In both instances, the veillonellae are metabolically coupled to their lactic acid producing coaggregation partners, growing at the expense of lactate produced from glucose fermentation.

Strains of Bacteroides gingivalis and Fusobacterium nucleatum occur in very high numbers in many cases of adult periodontitis and the former has been proposed by others to be a periodontal pathogen. Recent studies have shown that strains of these two organisms form stable coaggregates. Since F. nucleatum coaggregates with a large number of oral bacteria that are themselves capable of forming stable plaque consortia, the colonization and maintenance of B. gingivalis in the periodontal pocket can now be more easily rationalized.

A study of coaggregation-defective mutants of strains of Actinomyces naeslundii and Streptococcus sanguis is currently underway to more precisely enumerate and define the various types of adhesins on the surface of both gram positive bacteria. Because each interaction has distinctive properties, i.e., sugar sensitivity, it is possible to distinguish between the genotypically different mechanisms.

In an allied study, the adhesins of Bacteroides loescheii that mediate coaggregation with Streptococcus sanguis and Actinomyces israelii, respectively, were identified by preparing monoclonal antibodies against purified fimbrial preparations that agglutinated cells of both partners. Monoclonal antibodies were screened specifically for their ability to inhibit the coaggregation reactions. The clones that inhibited the B. loescheii - S. sanguis coaggregation recognized a 75kD polypeptide on immunoblots of fimbrial preparations that had been subjected to denaturing PAGE. In contrast, the clones that blocked the B. loescheii - A. israelii interaction reacted with a 45kD polypeptide in the same fimbrial preparations. Studies with coaggregation-defective mutants of B. loescheii indicated that the two adhesins were distinct proteins and that each was encoded by its own gene. Employing the appropriate monoclonal antibodies, immune electronmicroscopy revealed that the adhesins were not an integral part of the fimbriae, rather each adhesin was associated with the distal portion of the structure. They appeared to be arranged in clusters and were not associated with every fimbriae on the cell surface. The number of adhesin molecules per cell was estimated by binding studies conducted with the monoclonal antibodies. A theoretical maximum value of between 300 and 400 molecules per cell was obtained for each type of adhesin.

The adhesins have been purified and characterized using conventional and affinity chromatography. Characterization of the *B. loescheii* adhesin that mediates coaggregation with *S. sanguis* and also agglutinates erythrocytes represents one of the first physical descriptions of a bacterial lectin. In the native state, the adhesin is a hexamer composed of six 75kD subunits. Sufficient adhesin has been purified to determine the amino acid composition and N-terminal amino acid sequence of this protein. Unlike most proteins found on the surface of gram negative bacteria, this adhesin is exceedingly hydrophilic in nature and relatively basic (pI=8.2). The adhesin is currently being cloned in *E. coli* so that the complete amino acid sequence of the protein can be determined. The fimbriae that bear these adhesins are currently being purified and electron microscopic studies reveal a complex structure consisting of four helically arranged protein polymers. These structures appear to resemble the type 1 pili found in *Escherichia coli*.

In a parallel investigation, the outer membrane-associated adhesin of *Capnocytophaga gingivalis* that is responsible for its coaggregation with *Actinomyces israelii* has been identified and partially characterized. The approach that had been used successfully to identify and characterize the *B. loescheii* adhesins was also employed here. Monoclonal antibodies were screened for their ability to specifically inhibit the interaction between the two bacteria. These antibodies were used to demonstrate that the adhesin was a single polypeptide chain with a molecular mass of 230kD. The adhesin molecules which appear to be an integral part of the outer membrane were not uniformly located over the surface of the cell. Like the *B. loescheii* adhesins, they occured in clusters. The clusters appeared to be preferentially localized at the tips of the cells. Interestingly, it appears that not all cells in a given population possess the adhesin. The control mechanism that regulates the synthesis of the adhesin is currently under investigation.

The carbohydrate receptor on the surface of *Streptococcus sanguis* H1 that interacts with an adhesin on *Capnocytophaga ochracea* has been isolated, purified and characterized. Rhamnose, which was previously shown to be the most potent inhibitor of the reaction, is a major component of the repeating hexasaccharide that makes up this polysaccharide receptor. Mass spectroscopic analysis revealed that the sequence of sugars comprising the hexasaccharide are Rha-Rha-Gal-Gal-Gal-Glu. The hexasaccharides are linked via phosphodiester bonds. The nature of the linkage and anomeric forms of the sugars are being determined by NMR studies.

NIH LIBRARY

Innovative physiological studies on hexose metabolism of *Fusobacterium nucleatum*, another important coaggregation partner of many oral bacteria, have established that glucose and galactose are transported into the cell by a novel permease which recognizes only these two substrates. Energy for the transport of the sugars is derived from fermentation of the amino acids, glutamate, histidine and lysine. The sugars are not immediately metabolized following transport into the cells, but rather they are stored as glycogen. Only when the amino acid energy source is depleted, does the cell utilize the glycogen as an alternative energy and carbon source. Both the transport system and the catabolism of glutamate require chloride ion. Transport is also stimulated by ammonium ion. The role of chloride ion in transport and metabolism of this bacterium is under active investigation.

The newly discovered amino acids, N^5-(1-carboxyethyl) ornithine and N^6-(1-carboxyethyl) lysine, produced by *Streptococcus lactis*, have been structurally characterized. Chiral syntheses and NMR spectroscopy confirmed the structures and stereochemical configurations of the two amino acids. Studies with radiolabeled precursors established that the two compounds are produced by a reductive condensation of pyruvate and ornithine or lysine, respectively. The enzyme catalyzing this reaction, a hitherto undescribed synthetase, has been purified to electrophoretic homogeneity and is currently being characterized. Preliminary studies have shown that the enzyme has a molecular mass of 78kD and is composed of two 37-39kD subunits. Only ornithine and to a lesser extent, lysine, serve as substrates for the synthetase; NADP(H) is the required and specific cofactor. Pyruvate is the only keto acid that participates in the reductive condensation with the amino acids. The function of these two amino acids in this organism's metabolism is currently being investigated.

Continuing genetic and biochemical studies with gram positive oral bacteria are characterizing the plasmid and chromosomal genes responsible for the transport of lactose. The genes encoding Factor III^{lac} Enzyme II^{lac} and P-β-galactosidase were shown to constitute a functional operon and the genes encoding Factor III^{lac} and Enzyme II^{lac} have been cloned and sequenced. The latter enzyme was shown to consist of 577 amino acids and its structural characteristics have been compared to isologous enzymes found in streptococci and staphylococci. The arrangement of the three genes in the operon differs from the arrangement of the same genes in the staphylococcus lactose operon.

New techniques, including electroporation, are being developed to improve the efficiency of cloning of both homologous and heterologous genes in the lactic acid bacteria, *Lactobacillus casei* and *Lactobacillus bulgaricus*. The gene encoding the enzyme, α-amylase, from *Bacillus amyloliquifaciens* has been cloned into *L. casei*. Not only is the gene product expressed, but it is also exported from the cell into the medium indicating the lactobacillus recognized the signal sequence from the sporeformer. These studies are being aided by the development of new vectors and promotors for use with the oral and lactic acid bacteria.

Pathogenic Mechanisms Section

Our earlier investigations have demonstrated that certain oral actinomyces (e.g. A. viscosus T14V) possess two antigenically distinct types of fimbriae designated type 1 and type 2 and that other actinomyces (e.g. A. naeslundii WVU45) possess only type 2 fimbriae. These fimbriae have different functional properties; the type 1 mediate attachment to the tooth surface whereas the type 2 are associated with a lectin that recognizes receptors on other bacteria, epithelial cells and phagocytic cells. The genes for the subunits of two of these fimbriae (A. viscosus T14V type 1 and A. naeslundii type 2) have been cloned in E. coli by members of this Section and the gene for the A. viscosus T14V type 2 fimbriae has been cloned in collaboration with the Microbiology Section. The cloned subunits of all three fimbriae are of similar molecular weight as demonstrated by SDS-PAGE and immunoblotting with the relevant monoclonal and monospecific antibodies. The nucleotide sequences of these genes have now been determined and the amino acid sequences deduced from these data. Signal sequences of 30, 33 and 32 amino acids have been identified for the A. viscosus T14V type 1, A. viscosus T14V type 2 and A. naeslundii WVU45 type 2 fimbrial subunits, respectively. A protein of 533 amino acid residues is encoded by the gene for the A. viscosus T14V type 1 fimbrial subunit. The amino acid sequences of the intact fimbriae and the cloned subunit were identical through the first 26 residues as determined by Edman degradation. These sequences, in turn, were identical to the deduced amino acid sequence beginning at condon 31 of the cloned gene. The gene for the type 2 fimbrial subunit of A. viscosus T14V encodes a protein of 535 amino acids. The ribosome binding site and a putative transcription terminator have been localized within this gene. Since the production of this subunit by E. coli clones has been consistently low, recent efforts have focussed on enhancing its expression. This has been successfully accomplished by placing the gene adjacent to a promoter from bacteriophage T7 and introducing a plasmid, that allows the controlled expression of T7 RNA polymerase, into the E. coli clone. Antibodies have been raised against this subunit. Although they detect the same banding pattern on Western blots of intact fimbriae subjected to SDS-PAGE as antibodies against the intact fimbriae, their Fab fragments fail to inhibit the adherence of the actinomyces to certain oral streptococci. This finding strongly suggests that the lectin activity of these fimbriae may be due to a minor protein that is associated with them. The gene for the type 2 fimbrial subunit of A. naeslundii WVU45 encodes a protein of 531 amino acids. Residues 33-59 of the amino acid sequence deduced from the nucleotide sequence of this gene were identical to the 26 N-terminal residues of A. naeslundii WVU45 fimbriae. All three fimbriae and the cloned fimbrial subunits have been subjected to amino acid analyses. Excellent correlations have been obtained between these values and the amino acid compositions predicted from the nucleotide sequences of the respective genes.

With the acquisition of these data it has become possible to compare the primary structures of the three fimbrial subunits. Homology between the type 1 fimbrial subunit of A. viscosus T14V and the type 2 fimbrial subunit of either A. viscosus T14V or A. naeslundii WVU45 was 49%. Even more striking was the finding that homology between the two type 2 fimbrial subunits was 77%. These results clearly establish an evolutionary relationship between the genes for these three bacterial fimbriae.

Highly significant findings have been obtained concerning the receptors for these fimbriae on the tooth surface and on certain oral streptococci. It has now been firmly established that the acidic proline rich salivary proteins present in the acquired pellicle are specific receptors for the actinomyces type 1 fimbriae. Fractions of saliva that contain this protein and statherin, a structurally related protein, as well as purified acidic proline rich protein 1 serve as receptors whereas several other proteins examined were inactive. Conclusive evidence for the involvement of the type 1 fimbriae in these interactions was dependent on the selection of mutants of A. viscosus T14V that lack type 1, type 2 or both fimbriae. Only those strains possessing type 1 fimbriae adsorbed to hydroxyapatite beads coated with the acidic proline rich protein. The structures of the receptor molecules on two strains of streptococci (S. sanguis 34 and S. sanguis J22) for the lectin associated with the type 2 fimbriae of the actinomyces have been determined. The S. sanguis 34 receptor is a linear polysaccharide composed of hexasaccharide subunits with GalNAcβ13Gal at the reducing end while the S. sanguis J22 receptor is a polysaccharide consisting of branched heptasaccharide subunits with Galβ13GalNAc at the reducing ends. Emerging from these studies is the extremely interesting concept that these streptococcal receptor molecules are comprised of two distinct functional domains, one that is recognized by antibodies and the other by the bacterial lectin. Immunochemical studies utilizing potentially inhibitory saccharides have revealed that antibodies react with the non-reducing ends of the receptor subunits. The cross reactivity of the polysaccharides from the two streptococcal strains can be attributed to the structural similarity at the non-reducing ends of the subunits whereas the lack of immunological identity probably reflects the presence of a rhamnose branch on the S. sanguis J22 oligosaccharide. Saccharide inhibition studies have also demonstrated that the actinomyces lectin recognized the reducing end of the oligosaccharide receptor. We have previously found that Galβ13GalNAc and GalNAcβ13Gal also function as receptors for the bacterial lectin on mammalian cells. The common occurrence of these disaccharides in glycoconjugates on mammalian cells probably precludes their recognition as foreign antigens and antibodies are not, therefore, formed against these streptococcal lectin receptors.

New concepts concerning host defense against oral microorganisms have been developed based on findings obtained during the past year. Studies in one major area provide the first evidence that the interactions of lectins on different oral bacteria with their complementary receptors on polymorphonuclear leukocytes (PMNs) do not evoke identical PMN responses. The Gal/GalNAc reactive lectin associated with the type 2 fimbriae of certain strains of oral actinomyces (A. viscosus T14V and A. naeslundii WVU45) recognizes asialoglycoconjugates on PMNs following their exposure by sialidase, an enzyme produced by the bacteria. This recognition process results in the release of superoxide anion and the contents of secondary but not primary granules from the PMNs, stimulation of chemiluminescence, phagocytosis of the bacteria and, ultimately, bacterial destruction. These responses are inhibited by β-linked galactosides and bacterial mutants lacking the type 2 fimbriae fail to initiate any of these responses. A different pattern of PMN responses is elicited by a sialic acid reactive lectin on certain strains of oral streptococci (S. sanguis 34, S. sanguis 38 and S. sanguis DL1). The streptococcal lectin stimulates the release of superoxide anion and the contents of secondary granules from the PMNs. These responses are all inhibited by N-acetylneuraminlactose. In marked contrast to the actinomyces lectin, the interaction of the streptococcal lectin with PMN receptors does not result in killing of the bacteria. The streptococci are not inherently resistant to PMN dependent bactericidal activity since they are destroyed if they have been previously opsonized with antibody and complement. The streptococcal lectin does, however, mediate ingestion of the bacteria by the PMNs. Fluoresceinated bacteria are visualized within the PMNs under conditions that favor phagocytosis and this fluorescent pattern is not observed if N-acetylneuraminlactose is added to the incubation mixtures. This fluorescence cannot be quenched whereas the fluorescence of attached bacteria is readily susceptible to quenching. In addition to its inability to mediate bactericidal activity, the streptococcal lectin also fails to stimulate chemiluminescence, a response that is dependent on the production of singlet oxygen. Thus, PMN activation by the streptococcal lectin either occurs through a pathway different than that initiated by the actinomyces lectin or is terminated prior to the expression of bactericidal activity. These specific PMN responses have important implications in host defense and inflammation of host tissues. Whereas the actinomyces lectin can evoke PMN dependent inflammatory consequences it, in addition, provides protection of the host since the bacteria are destroyed. The streptococcal lectin, however, initiates inflammation but fulfills no function in host defense since the bacteria remain viable.

In addition to the sialo- and asialoglycoconjugate receptors on PMNs, oral bacteria can also interact with these phagocytic cells through the receptors for the Fc portion of immunoglobulin G (IgG) and receptors for cleavage products (C3b/C3bi) of the third component of complement. These latter receptors are of importance in an immune host in which the bacteria can be opsonized with IgG and C3b/C3bi. It has been assumed that any antibodies that react with the actinomyces can initiate Fc receptor dependent responses. Our investigations clearly demonstrate that this does not occur. IgG specific for the type 1 fimbriae of A. viscosus T14V and IgG reactive with the cell wall of these bacteria initiate Fc receptor mediated bactericidal activity. In contrast, IgG against the type 2 fimbriae was only minimally active at antibody concentrations thirty times greater than those of the other two antibodies. The inability of the anti-type 2 fimbriae IgG to initiate PMN dependent destruction of the bacteria could not be attributed to binding affinities or to the average number of antibody binding sites per bacterium. These antibodies were also used to deposit C3b/C3bi fragments on the bacteria which would then be available for interaction with their receptors. C3b/C3bi deposited during complement activation by the anti-cell wall IgG enhanced Fc receptor mediated bactericidal activity ten fold. The IgG specific for type 1 fimbriae was approximately twenty times less effective. Of major interest was the finding that complement activation by IgG against the type 2 fimbriae failed to enhance bacterial destruction despite the finding that this IgG was as effective as the IgGs of the other two specificities in depositing C3b/C3bi on the bacteria. These results are of major importance in defining host defense mechanisms that are operative against the oral actinomyces. Thus, antibody localization on specific bacterial structures determines susceptibility of the bacteria to Fc receptor mediated killing by PMNs. Furthermore, enhancement of PMN bactericidal activity by C3 fragments is strictly dependent on the site of deposition of these fragments which is, in turn, regulated by the antibody specificity.

PUBLICATIONS

Alpert C-A and Chassy BM. Molecular Cloning and nucleotide sequence of the factor III^{lac} gene of Lactobacillus casei. Gene 1988;62:277-288.

Boizet B, Flickinger JL, and Chassy BM. Transfection of Lactobacillus bulgaricus protoplasts by phage DNA. Appl Environ Microbiol. 1988;In press.

Calderone RA, Linehan L, Wadsworth E and Sandberg AL. Identification of C3d receptors on Candida albicans. Infect Immun 1988;56:252-258.

Chassy BM. Prospects for the genetic manipulation of lactobacilli. FEMS Microbiology Revs 1987;46:297-312.

Chassy BM and Flickinger JL. Transformation of Lactobacillus casei by electroporation. FEMS Microbiol Letters 1987;44:173-177.

Chassy BM, Flickinger JL and Mercenier A. Transformation of bacteria by electroporation. Trends in Biotechnology. 1987;In press.

Cisar JO, Brennan MJ and Sandberg AL. Bacterial and host cell receptors for the Actinomyces spp. fimbrial lectin. In Molecular Mechanisms of Microbial Adhesion. Hook M, Switalski L and Beachey EH (eds.) Springer-Verlag, New York. In press.

Cisar JO, Sandberg AL and Clark WB. Molecular aspects of oral microbial adherence and colonization. J Dent Res. In press.

Cisar JO, Sandberg AL, Yeung MK, Donkersloot JA, Chassy BM, Brennan MJ and Mergenhagen SE. Identification, characterization and functional properties of antigenically distinct fimbriae on Actinomyces. In Vaccines: New Concepts and Developments. Mohn JF (ed.) Longman, London. 1988;pp. 96-103.

Cisar JO, Vatter AE, Clark WB, Curl SH, Hurst-Calderone S and Sandberg AL. Mutants of Actinomyces viscosus T14V lacking type 1, type 2 or both types of fimbriae. Infect Immun 1988;In press.

Delisle AL, Donkersloot JA, Kolenbrander PE, and Tylenda CA. Use of lytic bacteriophage for Actinomyces viscosus T14V as a probe for cell surface components mediating intergeneric coaggregation. Infect Immun 1988;56:54-59.

Gibbons RJ, Hay DI, Cisar JO, and Clark WB. Adsorbed salivary proline-rich protein-1 and statherin are pellicle receptors for type 1 fimbriae of Actinomyces viscosus T14V-J1 on

apatitic surfaces. Infect Immun 1988;In press.

Hughes CV, Kolenbrander PE, Andersen RN and Moore LVH. Coaggregation properties of human oral *Veillonella*: relationship to colonization site and oral ecology. Appl Env Microbiol 1988;In press.

Kolenbrander PE. Intergeneric-coaggregation among human oral bacteria and ecology of dental plaque. Annu Rev Microbiol 1988;In press.

Kolenbrander PE. Surface recognition among oral bacteria: multigeneric coaggregations and their mediators. CRC Critical Reviews in Microbiology. 1989;In press.

Kolenbrander PE and Andersen RN. Intergeneric rosettes: sequestered surface recognition among human periodontal bacteria. Appl Env Microbiol 1988;54:1046-1050.

London J, Weiss EI and Kolenbrander PE. Adhesins of *Bacteroides* *loescheii*. *In* Molecular mechanisms of microbial adhesion. Beachey EH, Hook M, and Switalski LM (eds.) Springer-Verlag, New York. 1988;In press.

McIntire FC, Crosby LK, Vatter AE, Cisar JO, McNeil MR, Bush CA, Tjoa SS and Fennessy PV. A polysaccharide from *Streptococcus* *sanguis* 34 that inhibits coaggregation of *S. sanguis* 34 with *Actinomyces* *viscosus* T14V. J Bacteriol 1988;170:2229-2235.

Mercenier A and Chassy BM. Strategies for the development of bacterial transformation systems. Biochimie 1988;In press.

Mergenhagen SE, Sandberg AL, Chassy BM, Brennan MJ, Yeung MK, Donkersloot JA, and Cisar JO. Molecular basis of bacterial adhesion in the oral cavity. Rev Infect Dis 1987;9:S467-S474.

Miller SPF and Thompson J. Biosynthesis and stereochemical configuration of N^5-(1-carboxyethyl) ornithine. An unusual amino acid produced by *Streptococcus lactis*. J Biol Chem 1987;262:16109-16115.

Porter EV and Chassy BM. Nucleotide Sequence of the ß-D-Phosphogalactoside galactohydrolase gene of *Lactobacillus casei*: Comparison to ß-D-phosphogalactoside galactohydrolase genes of other gram-positive organisms, Gene 1988;62:263-276.

Reizer J, Deutscher J, Grenier F, Thompson J, Hengstenberg W and Saier MH, Jr. The phosphoenolpyruvate: sugar phosphotransferase system in Gram-positive bacteria: properties, mechanism and regulation. CRC Microbiol Rev 1988;15:297-338.

Robrish SA, Oliver C, and Thompson J. Amino acid-dependent transport of sugars by *Fusobacterium nucleatum* ATCC 10953. J Bacteriol 1987;169:3891-3897.

Robrish SA and Thompson J. Suppression of polyglucose degradation in *Fusobacterium nucleatum* 10953 by amino acids. FEMS Microbiol Letts 1988;In press.

Sandberg AL, Mudrick LL, Cisar JO, Metcalf JA and Malech HL. Stimulation of superoxide and lactoferrin release from polymorphonuclear leukocytes by the type 2 fimbrial lectin of *Actinomyces viscosus* T14V. Infect Immun 1988;56:267-269.

Shimizu-Kadota M, Flickinger JL, and Chassy BM. Evidence that the *Lactobacillus casei* insertion element, ISL1, has a limited host range. J Bacteriol 1988;In press.

Thompson J. Ornithine transport and exchange in *Streptococcus lactis*. J Bacteriol 1987;169:4147-4153.

Thompson J. Regulation of sugar transport and metabolism in lactic acid bacteria. FEMS Microbiol Rev 1987;46:221-231.

Thompson J. Lactic acid bacteria: model systems for *in vivo* studies of sugar transport and metabolism in Gram-positive organisms. Biochimie 1988;70:325-336.

Thompson J and Miller SPF. N^6-(1-Carboxyethyl)lysine formation by *Streptococcus lactis*. Purification, synthesis, and stereochemical structure. J Biol Chem 1988;263:2064-2069.

Weiss EI, Kolenbrander PE, London J, Hand AR and Andersen RN. Fimbria-associated proteins of *Bacteroides loescheii* PK1295 mediate intergeneric coaggregations. J Bacteriol 1987;169:4215-4222.

Weiss EI, London J, Kolenbrander PE, Andersen RN, Fischler C and Siraganian RP. Characterization of monoclonal antibodies to fimbria-associated adhesins of *Bacteroides loescheii* PK1295. Infect Immun 1988;56:219-224.

Weiss EI, London J, Kolenbrander PE, Hand AR and Siraganian RP. Localization and enumeration of fimbria-associated adhesins of *Bacteroides loescheii*. J Bact 1988;170:1123-1128.

Yeung MK and Cisar JO. Cloning and nucleotide sequence of a gene for *Actinomyces naeslundii* WVU45 type 2 fimbriae. J Bacteriol 1988;In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00042-18 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biological Characterization of Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce M. Chassy	Research Chemist	LMI	NIDR
Jeannette Flickinger	Microbiologist	LMI	NIDR
Carl-Alfred Alpert	Visiting Associate	LMI	NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to use genetic, biochemical and physiological approaches to investigate the pathogenicity of oral bacteria. The two specific areas of investigation are: 1) characterization of plasmid-coded and chromosomal metabolic genes from oral bacteria and 2) development of systems for genetic exchange in oral bacteria. The nucleotide sequence of the EnzymeIIlac gene of Lactobacillus casei was completed. It displayed high homology to the same genes isolated from staphylococcus aureus and Streptococcus lactis. Little homology was observed in comparison with other EnzymeII found in Escherichia coli, Salmonella typhimurium or Bacillus subtilis. The genes encoding the EnzymeIIlac, P-β-gal and Factor IIlac were found to comprise an operon-like structure. Site-directed mutagenesis has been used to prepare mutant EnzymeII proteins with altered catalytic activity. The sequence of the plasmid-coded β-galactosidase of Lactobacillus casei has been completed: the adjacent lac permease gene has been cloned and its sequence is being determined. L. casei has been utilized for the cloning and expression of heterologous genes such as α-amylase, subtilisin and β-gal. Expression and secretion of foreign proteins has been observed; improved vectors and promoters have been identified. Using electroporation, L. bulgaricus and L. acidophilus have been transformed. The feasibility of use of these food related bacteria that colonize the gastrointestinal system as vectors for gene delivery to humans is being examined.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE00043-18

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological and Genetic Studies on Pathogenic Oral Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

Dr. Donkersloot, Jacob	Research Microbiologist	LMI	NIDR
Mr. Harr, Robert	Biology Lab. Technician (Micro)	LMI	NIDR

COOPERATING UNITS *(if any)*
Dr. R.L. Cihlar, Georgetown University School of Medicine
Dr. A.L. Delisle, University of Maryland Dental School

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	.90	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A chromosomal gene (fimA) which encodes a subunit of type 2 fimbriae of Actinomyces viscosus T14V has been sequenced. The results revealed an open reading frame of 1605 nucleotides encoding a protein of 56 kDa. The (ATG) initiation codon is preceded by a strong consensus ribosome binding site located 13 nucleotides upstream. A putative leader sequence of 33 amino acids has been identified; hence, the mature subunit (p53) is 53 kDa in size. Translation is terminated by two (TGA) stop codons and a putative (rho-dependent) transcription terminator is located 25 nucleotides further downstream. Analysis of codon usage showed a strong preference for C in the third position. The amino acid composition of the mature subunit as deduced from the nucleotide sequence agreed well with the chemical analysis of affinity-purified type 2 fimbriae. Comparison of the primary amino acid sequence with that of the subunit of A. naeslundii WVU45 (type 2) fimbriae indicated a surprising degree of similarity (77%), especially considering the fact that polyvalent and monoclonal antibodies against these fimbriae barely cross-react. It appears likely that antigenic sites on these fimbriae have diverged rapidly under the immunological pressure of salivary antibodies in the mouth. A much lower degree of similarity (49%) was noted between type 1 and type 2 fimbriae of A. viscosus T14V.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00061-17 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Complement Activation and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ann Sandberg	Chief, Pathogenic Mechanisms Section	LME NIDR
Caron Lyman	Staff Fellow	LME NIDR
Richard Joralmon	Nat'l Res. Ser. Award	LME NIDR
Linda Mudrick	Microbiologist	LME NIDR
John Cisar	Research Microbiologist	LME NIDR
Chieri Kurashima	Visiting Associate	LME NIDR

COOPERATING UNITS *(if any)*

Dr. Al Vatter, University of Colorado

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Pathogenic Mechanisms Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are continuing to define the molecular basis for the interaction of oral bacteria with human polymorphonuclear leukocytes (PMNs) and to examine the consequences of these recognition processes. A Gal/GalNAc reactive lectin associated with the type 2 fimbriae of certain oral actinomyces initiated phagocytosis and killing of the bacteria by PMNs. This process was accompanied by the production of superoxide anion and the release of the contents of the PMN secondary granules. The interaction of a sialic acid reactive lectin on certain strains of oral strep-tococci evoked a different pattern of PMN responses. This lectin also stimulated superoxide anion production by the PMNs and elicited the release of the contents of secondary granules. However, unlike the actinomyces lectin, the streptococcal lectin did not initiate PMN dependent bactericidal activity although its interac- tion with complementary PMN receptors did result in ingestion of the bacteria as determined by fluorescence microscopy. The actinomyces and streptococcal lectins also differed in their abilities to stimulate PMN chemiluminescence. This res- ponse was initiated by the actinomyces lectin but not by the streptococcal lectin. Thus, the sequence of PMN activation events induced by the two bacterial lectins are not identical. The receptors for the Fc portion of immunoglobulin G (IgG) and the receptors for fragments (C3b/C3bi) of the third component of complement are involved in the interaction of opsonized bacteria with PMNs. Studies utilizing IgGs raised against different structures (cell surface, type 1 fimbriae or type 2 fimbriae) on oral actinomyces demonstrated IgGs against the bacterial surface and type 1 fimbriae initiated bactericidal activity whereas those specific for the type 2 fimbriae were ineffective. Enhancement of Fc receptor mediated bacterici- dal activity by C3b/C3b; only occurred when these fragments were directed towards the cell surface. These findings demonstrate that PMN mediated host defense against these oral actinomyces is strictly dependent on the localization of IgGs and C3 fragments on certain bacterial structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00273-10 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell-Cell Interactions Between Oral Actinomyces and Othr Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Kolenbrander, Paul	Reseacrch Microbiologist	LMI	NIDR
Dr. Andersen, Roxanna	Microbiologist	LMI	NIDR
Dr. Hughes, Christopher	Dental Staff Fellow	LMI	NIDR
Dr. London, Jack	Research Microbiologist	LMI	NIDR
Dr. Donkersloot, Jacob	Research Microbiologist	LMI	NIDR
Dr. Siraganian, Reuben	Chief, Clinical Immunology	LMI	NIDR

COOPERATING UNITS *(if any)* Dr. A.L. Delisle, U. of Maryland, School of Dentistry, Baltimore, MD; Dr. L.V.H. Moore, VPI and SU, Blacksburg, VA; Dr. C. Tylenda, CIPCB, NIDR; Dr. A. Hand, CIPCB, NIDR; Dr. E. Weiss, Tel Aviv University, Tel Aviv, Israel.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.15	2.15	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As additional information regarding cell-to-cell recognitions among oral bacteria is discovered, it is becoming increasingly clear that a dynamic but organized microbial community exists in the oral cavity. The part of this complex community studied in this laboratory pertains especially to microbial ecology. Both gram-positive and gram-negative bacteria are tested as coaggregation partners. The concept of highly specific partnerships advanced by this laboratory gained additional support with the evidence that strains of Bacteroides gingivalis, a presumed periodontal pathogen, coaggregates with Fusobacterium nucleatum and Eubacterium nodatum strains, that are also associated with periodontal disease, but not with any other oral strains. Some coaggregations are inhibited by lactose, which adds to the growing number of sugar-inhibitable pairings among the oral bacteria.

An intensive survey of strains of Veillonella from the tongue, buccal mucosa, and saliva revealed that veillonellae from the tongue coaggregate with Streptococcus salivarius, also a tongue inhabitant, but not with subgingival bacteria. Whereas, veillonellae from subgingival plaque coaggregate with other subgingival bacteria but not with S. salivarius. These results provide the first direct evidence that coaggregation is tightly coupled to the ability of a strain to colonize a specific econiche.

The results of each of these investigative approaches are focused on understanding the relationship of cell surface recognitions among oral bacteria and their role in microbial ecology.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00341-07 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of sugar transport and metabolism in lactic acid and oral bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thompson, John	Visiting Scientist	LMI	NIDR
Robrish, Stanley	Research Microbiologist	LMI	NIDR

COOPERATING UNITS *(if any)*

Stephan P. F. Miller, Dev. and Metabolic Neurology Branch, NINCDS, NIH

LAB/BRANCH

Laboratory of Microbiology and Immunology, NIDR

SECTION

Microbiology Secton

INSTITUTE AND LOCATION

National Institute of Dental Research, 30/522, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Two previously unknown amino acids have been identified, and purified from cells of Group N streptococci. The chemical structures and stereochemical configurations of N(5)-(1-carboxyethyl)ornithine and N(6)-(1-carboxyethyl)lysine have been confirmed by chiral syntheses and NMR-spectroscopy.
2. It has been established that the in vivo synthesis of these unusual compounds occurs via the NAD(P)H-dependent reductive condensation between pyruvic acid and the side-chain amino groups of ornithine and lysine. The synthase responsible for the biosynthesis of these novel amino acids has been purified (8000-fold) to homogeneity from cells of Streptococcus lactis K1. The physical and enzymatic properties of the enzyme have been partially defined.
3. The accumulation of sugars (glucose and galactose) by cells of Fusobacterium nucleatum 10953 is dependent upon the provision of glutamic acid, lysine or histidine. These fermentable amino acids provide the energy necessary for sugar transport, phosphorylation and synthesis of endogenous sugar polymer(s). The presence of the amino acids also inhibits the degradation of pre-formed polymer, but in the absence of glutamate, lysine or histidine endogenous polymer is fermented via the Embden-Meyerhof pathway. The concentrations of Na^+ and NH_4^+ ion in the environment markedly affect the rates of sugar transport by resting cells of F. nucleatum. The biochemical roles of

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 382-05 LMI

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Growth and Interaction of Oral Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robrish, Stanley A.	Research Microbiologist	LMI	NIDR
Thompson, John	Visiting Scientist	LMI	NIDR
Gomez, Irma	Microbiology	LMI	NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.10	1.10	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The carbohydrate storage product made from glucose or galactose by F. nucleatum proved to be a polyglucose or glycogen. The use of the glycogen, made from either glucose or galactose, was spared when energy was available to the washed cells by fermenting amino acids. Glutamate, lysine and histidine, the amino acids previously shown to stimulate sugar transport, were the same ones which anaerobically suppressed glycogen use. Aerobically the amino acids had no effect on the loss of label from glycogen previously synthesized from glucose or galactose. Analysis of intermediates during glycogen use revealed that glucose was used by the Embden-Meyerhoff pathway by an organism which lacks a phosphoenolpyruvate phosphotransferase sugar transport system. Analysis of fermentation products obtained from cells using labeled preformed glycogen revealed that butyric acid was a product of the fermentation of sugars as well as amino acids. Washed cells from a culture of F. nucleatum, grown on a sugar free complex medium, were able to transport glucose or galactose without a lag if the anaerobic cell suspension was incubated with glutamate before the addition of the sugar.

Sodium is important in the glutamate mediated stimulation of glucose transport and/or, glycogen synthesis by F. nucleatum. The ammonium salt of glutamic acid would not effectively stimulate glucose or galactose use unless sodium chloride was included in the incubation mixture. In addition, ammonium glutamate would not be used anaerobically by a cell suspension of F. nucleatum without sodium chloride.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00454-02 LMI

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Surface Molecules in Metabolism and Ecology of Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

London, Jack	Research Microbiologist	LMI	NIDR
Allen, Janet	Microbiologist	LMI	NIDR
Cassels, Fred	Warner-Lambert Fellow	LMI	NIDR
Citron, Jean	Staff Fellow	LMI	NIDR
Kolenbrander, Paul	Microbiologist	LMI	NIDR
Siraganian, Reuben	Chief, Clinical Immunology Section	LMI	NIDR

COOPERATING UNITS *(if any)* H. Fales, LC, NIHLBI; A. Hand, PICPB, NIDR; P. Tempro, U. of Conneticut; E.I. Weiss, U. of Tel-Aviv; D. Zopf, LP, NCI

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.05	2.05	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The two fimbriae associated adhesins of Bacteroides loescheii which mediate the coaggregations with Streptococcus sanguis 34 or Actinomyces israelii PK14 are presently being characterized. The S. sanguis 34 specific adhesin appears to be a hexamer composed of 6 - 75kD subunits. Moderate quantities of this protein have been purified to homogeneity by conventional and affinity chromatography. Its N-terminal amino acid sequence and amino acid composition has been determined and its pI has been estimated. Purification and characterization of the A. israelii PK14 adhesin is also underway. A method of purify the fimbriae that carry these adhesins has been developed, these larger structures appear to be complex helices composed of four intertwined protein strands.

The carbohydrate receptor on S. sanguis H1 which is recognized by an adhesin on Capnocytophaga ochracea has been isolated from streptococcal cell walls, purified to homogeneity and characterized by NMR and mass spectroscopy. It was shown to be composed of a repeating hexasaccharide composed of rham-rham-gal-gal-gal-glu linked through phosphodiester groups.

Monoclonal antibodies specific for an afimbriate adhesin on the outer membrane of Capnocytophaga gingivalis DR2001 have been used to identify and localize this lectin like protein. The adhesin mediates the coaggregation by C. gingivalis and strains of Actinomyces israelii. The adhesins are not found uniformly over the surface of the cell and appear to be preferentially located at the poles of the cell. Like B. loescehii, there are relatively few adhesin molecules per cell, between 190 and 300.

ANNUAL REPORT OF THE NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch utilizes a multidisciplinary approach that includes anatomical, pharmacological, biochemical, physiological, behavioral and psychophysical techniques to study neural function as it relates to the processing of sensory signals about tissue-damage. Correlative approaches are often used to answer questions about the functional organization of nociceptive systems in normal and pathological states. The clinical component of the Branch develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of mechanisms of acute and chronic pain in humans as well as to the evaluation of new techniques for the treatment of pain.

This year we have continued to emphasize the role of neurochemical messengers in the organization and function of pathways in the brain involved in transmitting information about pain. Our previous studies of the organization of these systems in normal states have provided a foundation for recent studies on endocrine and neural function following the development of tissue inflammation and after nerve injury. We have developed and utilized new models of peripheral tissue damage and nerve injury and have focused on changes occurring at the site of inflammation as well as in the central nervous system. Our animal studies provide a conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. In our correlative studies of behavior and neural function in awake monkey, we have examined the role of the medullary dorsal horn and the cerebral cortex in pain transmission in the normal state.

The Branch continues to provide the leadership and coordination of the activities of the program on clinical pain research at the NIH. This includes collaborative studies with staff of a number of other institutes including NCI, NIMH, NHLBI, the Clinical Center, and the Naval Medical Center.

Investigators in the Branch have received considerable recognition for their research accomplishments. Four investigators in the Branch were invited speakers at a NATO Advanced Research Workshop on the superficial dorsal horn held in El Escorial, Spain. Dr. Bennett was one of the organizers of this international workshop. Dr. Ruda participated in the exchange program established between NIDR and CINVESTAV (Center for Research and Advanced Studies, Mexico) and spend one month in Mexico City collaborating with neuroscientists studying spinal cord function. This collaboration has continued and a graduate student from the Neurosciences Program of CINVESTAV is presently conducting research in the Branch. Dr. Iadarola has received considerable recognition for his recent studies and will chair a symposium at the upcoming Society for Neuroscience meeting in Toronto. Dr. Dubner is President of the American Pain Society this year and is a member of the governing council and the scientific program committee of the International Association for the Study of Pain. In addition, he was the recipient of the Wolstencroft Memorial Lectureship at the recent Brain Research Association meeting in London.

Research accomplishments of the Branch are presented in detail below.

Neurochemistry of Nociceptive Pathways in Normal and Pathological States

With the use of immunocytochemical methods, retrograde markers, and techniques from molecular biology, we continue to determine the neurochemical mediators associated with the major components of the medullary and spinal dorsal horns: the primary afferents, the intrinsic dorsal horn neurons, and extrinsic afferent inputs originating from distant sites. This year we have continued to study the role of the peptides, calcitonin gene-related peptide (CGRP) and substance P (SP), in dorsal horn function, utilizing immunocytochemical methods. Since CGRP appears to arise exclusively from primary afferents, the co-localization of CGRP and SP to the same axons can be used to identify SP primary afferent axons. Neonatal capsaicin, a selective neurotoxin, has been used to destroy small primary afferents and to examine the effects of such lesions on nocifensive behavior and neural plasticity following neonatal nerve lesions. Previous studies have shown a time-dependent return of thermal sensitivity and partial recovery of spinal CGRP after neonatal capsaicin treatment. Experiments were designed to utilize the colocalization of SP and CGRP to determine the effect of neonatal capsaicin on SP primary afferent axons. In two-week old animals, few co-localized axons remained in Lissauer's tract and laminae I, II and V as compared to controls. AT 12-16 weeks, there was an increase in the number of co-localized axons in these areas. There also was a reappearance of CGRP axons traversing laminae III and IV which did not co-localize SP. The data suggest that neonatal capsaicin depletes primary afferent SP but does not completely destroy all axons and that there is a reappearance of axons as early as 3-4 weeks. These findings are of importance because of the role of substance P in nociceptive function in the dorsal horn.

The presence of CGRP in small myelinated and unmyelinated axons was used as a marker to determine the rostral projections of such axons in the spinal cord. Rhizotomies were performed in five consecutive segments of the lumbar and sacral dorsal horn on one side. For controls, a few animals received an ipsilateral hemisection in the most rostral rhizotomized segment to remove descending axons. CGRP-like immunoreactivity was greatly reduced, but not eliminated, in all rhizotomized segments compared to the intact contralateral side. Preliminary results from an electron microscopic analysis show that all the remaining CGRP-like immunoreactive axons in Lissauer's tract were unmyelinated. Our interpretation of these results is that most of the remaining CGRP originated in caudally located intact dorsal roots and that small diameter primary afferents project at least five segments rostral to their segment of entry.

This year we have continued to study the effects of peripheral tissue injury and inflammation. Inflammation is a common component of many acute and chronic pain conditions including postsurgical acute pain, cancer pain and arthritis. We have developed two models of inflammation. An acute carrageenan model of inflammation mimics acute pain produced by tissue damage from surgery. This model allows us to directly relate animal findings to our clinical model of postsurgical pain following the extraction of impacted third molars (see below). The carrageenan model is highly predictive for detecting drugs that possess analgesic and anti-inflammatory activity in humans and this is an appropriate model to parallel the clinical studies. With this model we have examined endocrine changes at the site of peripheral inflammation and have correlated these changes with behavioral hyperalgesia and tissue components of the inflammatory process. In a second model of inflammation, we have looked at changes in spinal dorsal horn neuropeptides associated with a more persistent hyperalgesia lasting at least 5 days. This model may be useful in examining neural changes associated with more persistent models of pain in humans such as cancer pain and arthritis.

This year we have focused on the role of the bradykinin system in mediating human and animal conditions of inflammation and have examined the potential clinical utility of bradykinin antagonists. One approach has been to evaluate the efficacy of bradykinin analogs which display receptor antagonist properties. Three different antagonists reduced carrageenan-induced hyperalgesia as well as the edema associated with the inflammation. The results indicate that bradykinin partly mediates carrageenan-induced inflammation and that effective receptor antagonists may have clinical utility.

Another important finding on the role of endocrine changes at the site of peripheral tissue injury comes from studies on activation of the pituitary-adrenal axis. Our research over the last two years indicates that circulating levels of beta-endorphin, a peptide product of one of the three major families of opioid peptides, may modulate the perception of pain. Beta-endorphin is released from the pituitary and its production is stimulated by corticotrophin releasing factor (CRF). Our present studies show that there is a peripheral corticotroph stimulating factor in inflamed tissues that stimulates beta-endorphin release in both denervated rats (thus ruling out a neural initiation of hypothalamic CRF) and in cell cultures. Our findings raise the possibility that the pituitary-adrenal response to a stressor is a result of both hypothalamic and extra-hypothalamic input, with stressors differing in their activation of these two systems.

Inflammation also results in central nervous system changes that involve the activation of specific opioid peptides. We have shown that inflammation induced by Freund's adjuvant, yeast, phorbol ester, or carrageenan, results in significant increases in the dorsal horn content of the opioid peptide, dynorphin, and a rapid increase in dynorphin mRNA. These data demonstrate that the increase is a common feature of inflammation and is not unique to any one inflammatory agent. The increase in synthesis of opioid peptide is mainly confined to the dynorphin family since none of the test agents produced a major alteration in enkephalin mRNA or peptide. In-situ hybridization histochemistry, using an oligomer complementary to a portion of preproenkephalin mRNA, demonstrated labeled cells in virtually all spinal cord laminae. However, we could not detect an increase in enkephalin mRNA on the side of the spinal cord receiving inputs from the inflamed limb. These observations suggest that the two intrinsic spinal opioid systems which originate from two different genes are acting differentially in models of peripheral inflammation and hyperalgesia.

A series of experiments has been initiated to examine the mechanisms of dynorphin gene regulation associated with peripheral tissue injury. The time course of the expression of the proto-oncogene c-fos has been studied. C-fos is a nuclear protein that is involved in the transcriptional control of gene expression. There is a rapid and transient increase in c-fos mRNA in the spinal cord during the inflammation. An antibody to a portion of the c-fos molecule has been raised and we are assessing alterations in the production of c-fos protein using western blot analysis. These studies will provide information about the factors involved in the initiation of dynorphin gene expression and its relationship to second messenger systems linked to primary afferent transmitters activated during inflammation (e.g., SP and CGRP).

We have examined the relationship between dynorphin-containing neurons and CGRP in the inflammation model using immunocytochemical double-labeling techniques. CGRP immunoreactive varicosities were observed contacting about half of the dynorphin immunoreactive neurons in laminae I, II and V of the dorsal horn ipsilateral to the inflammation. The contacts were located on both the cell somata and proximal dendrites and typically numbered greater than 25 per cell. These findings suggest that there are direct contacts between presumed nociceptive primary afferents and opioid-containing neurons which exhibit an upregulation of dynorphin as a result of peripheral inflammation and hyperalgesia.

By using a combination of retrograde transport markers and immunocytochemical labeling, we have identified spinal projection neurons that contain dynorphin following peripheral inflammation. There is a proportionally greater increase in the number of dynorphin-containing neurons in lamina I than in lamina II after the induction of peripheral inflammation. This increase is more pronounced in lamina I neurons projecting to the midbrain than those not identified as projection neurons, a result of the presence of dynorphin-containing neurons in the more dorsal part of laminae I where the projection neurons are found. These findings are of interest because they suggest that the dynorphin changes in the dorsal horn following inflammation may have direct effects in more rostral portions of the brain where lamina I neurons project as opposed to an effect limited to local spinal circuits.

We have studied the physiology of the neurons in the superficial spinal dorsal horn in the same models of inflammation in order to examine changes in the physiological properties of the neurons associated with the behavioral hyperalgesia. The distribution of receptive field size for cells in the superficial laminae of the dorsal horn following peripheral inflammation included many large receptive fields, often involving the entire surface of the ipsilateral paw. Many cells exhibited complex responses including discontinuous fields, regions with different thresholds, and responses to deep structures or joint movement. Most cells demonstrated spontaneous activity. All of the above findings are rare in normal animals with uninflamed paws. These effects were seen within 6 hours of the injection of the inflammatory agent. We have also recorded the activity of dorsal root ganglion cells in control animals and after peripheral inflammation. Dorsal root ganglion cells recorded ipsilateral to inflamed hindpaws had receptive fields and mechanical thresholds very similar to those of cells recorded in control animals. These data provide evidence that a population of lamina I nociceptive projection neurons demonstrates increased activity in a model of inflammation and hyperalgesia. This increased responsiveness may be associated with a range of pain sensations, including pain in the absence of stimulation of the affected part, and referred pain. The expanded receptive fields of these neurons does not appear to be accounted for by peripheral sensitization of receptors and therefore may involve more central mechanisms.

In order to examine the effects of nerve injury on neuronal activity and behavior, we have developed a model of hyperalgesia and allodynia in rat. This is the first model in animals that appears to adequately mimic neuropathic pain in humans with nerve injuries due to trauma or disease. The nerve injury produces large changes in the neurochemistry of small diameter, primary afferent neurons whose axons travel in the injured nerve. The changes begin within 5-10 days and persist for 2-3 months. There are decreases in the levels of SP, CGRP and fluoride-resistant acid phosphatase in the dorsal root ganglia and also in terminal axonal arbors in spinal dorsal horn laminae I-II and V. In addition, there is an increase in the levels of dynorphin peptide in the dorsal horn. This increase is initially found in neurons in laminae I and V-VI, but by 10 days, the increase can only be detected in the deeper laminae. Nerve function has been studied one day after the injury and a large majority of the myelinated axons cannot conduct impulses across the injured region. However, nearly all of the unmyelinated fibers conduct normally. About 25% of the myelinated axons exhibit spontaneous, ectopic discharges. All of these observations indicate that nerve injury evokes pathophysiological processes in both the peripheral and central nervous systems. The relationship of these changes to the behavioral signs of pain is an area of future study.

Neural Mechanisms of Pain at Dorsal Horn and Cerebral Cortex Levels

In previous years we developed a thermal discrimination task to examine whether monkeys can make fine thermal discriminations in the noxious heat range. With this behavioral model, we studied the role of different neurons in the medullary dorsal horn in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. In addition, we employed this model to examine the effect of opioid drugs on the perceived intensity of such stimuli. This year we have continued these studies in three ways. First, we have studied further the participation of classes of neurons in the medullary dorsal horn in the monkeys' ability to perceive the intensity of noxious heat. Second, we have studied the effect of noradrenergic systems on the perceived intensity of noxious heat stimuli in the task. Third, we have examined the role of the cerebral cortex in pain by studying the response properties of neurons in somatosensory cortex while monkeys performed this task.

Our findings argue for the importance of wide-dynamic-range neurons in the medullary dorsal horn in the detection and discrimination of noxious heat stimuli. The ability of monkeys to perform successfully in thermal detection tasks, determined from threshold responses and detection speeds, is dependent on the activity of a subclass of WDR neurons. The neuronal discharge of nociceptive-specific neurons alone cannot provide the minimal information necessary to account for the monkeys' ability to detect increases in noxious heat intensity in the 44° to 47°C range. The similar detection speeds in monkeys and humans, and the high correlation of detection speed with the perceived intensity of these same noxious heat stimuli in human subjects, supports the conclusion that WDR neurons participate in neural coding mechanisms leading to the perception of pain.

Activation of descending noradrenergic systems exerts powerful inhibitory effects on the transmission of nociceptive information. We examined the effects of a selective alpha-2 adrenergic agonist, ST-91, on the monkeys' ability to perceive the intensity of noxious heat stimuli. ST-91 was directly microinjected into the medullary dorsal horn where the terminals of descending noradrenergic fibers are located. We found that ST-91 strongly attenuated the monkeys' ability to perceive the intensity of noxious heat stimuli. These effects were dose and stimulus-intensity dependent. The detection of either visual or cooling cues was not modified by any of the doses of ST-91. These results show that ST-91 microinjected into the medullary dorsal horn impairs the detection of noxious heat stimuli without altering motor function, motivational or attentional variables. These findings indicate that alpha-2 adrenergic agonists, similar to morphine, attenuate the sensory-discriminative capacities of monkeys to detect noxious heat stimuli, when injected directly into the dorsal horn. Future studies need to evaluate the pharmacological specificity of the effect and to evaluate the possibility of combining opiates and noradrenergic agonists in the control of pain.

The role of the primary somatosensory cortex in pain remains unresolved. We have attempted to determine its role in pain by training monkeys to detect small increases in noxious heat intensity. To date, 36 somatosensory cortex (SI) neurons have increased their discharge frequency to noxious thermal stimuli applied to the face. Thirty-four of these neurons were classified as wide-dynamic-range and two neurons were classified as nociceptive-specific. Twenty-two neurons increased their discharge frequency in a graded fashion to increasing intensities of noxious heat. The remainder did not grade their response in a systematic fashion with the intensity of the stimulus. Activity from 15 cortical nociceptive neurons was studied while the monkeys responded to small stimuli of 0.2 to 0.8°C from a baseline of 45° or 46°C. A linear regression of individual trials revealed a significant relationship between neuronal discharge and behavioral detection speed. These data provide the first demonstration that in the awake monkey there are two populations of cortical nociceptive neurons that respond to noxious thermal stimulation. One population of neurons encodes small changes in noxious heat intensity and may participate in the monkeys' ability to detect temperature changes in the noxious range. The other population of neurons does not encode the intensity of noxious thermal stimulation, but is more suited for a role in arousal or alerting responses which are a consequence of noxious thermal stimulation. These results put to rest the long held view that the cerebral cortex does not participate in the encoding of stimulus features in the noxious range.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in mammalian cerebral cortex. We have used immunocytochemical methods to study the distribution of GABA in somatosensory areas that may play a role in pain transmission. In one study, the distribution of neurons immunoreactive for glutamic acid decarboxylase (GAD) was examined in the first (SI) and second (SII) somatosensory cortex. GAD immunoreactive somata and axons were present in all layers of SI and SII, but were most numerous in layer IV of SI and in layer III of SII. In a second study, the distribution of GABA immunoreactivity was examined in the retroinsular cortex (Ri) and area 7b of the cerebral cortex. GABA immunoreactivity was observed in all layers, but the majority of labelling was found in the superficial cortical layers. These studies provide information on the distribution of inhibitory mechanisms across different regions of the cerebral cortex and will help our basic understanding of how somatosensory information is processed and modified in the central nervous system.

The Assessment of Experimental and Acute Clinical Pain

The purpose of these human pain studies is to develop psychophysical and behavioral models of pain perception in humans and to utilize these models in the understanding of pain mechanisms in humans and the development of new methods of pain control. A new scaling method of pain assessment was developed last year that provides temporal information about the effects of analgesic agents on the intensity of painful stimuli. This year the sensitivity of the interactive staircase procedure was used to assess the effects of fentanyl and its reversal by naloxone. The initial infusion of fentanyl, in comparison to placebo, significantly increased the stimulus intensities required to evoke the same pain responses. Naloxone reduced these stimulus intensities when it followed the administration of fentanyl. Interestingly, there was a nonsignificant trend for naloxone alone to increase pain sensitivity in comparison to placebo. These findings indicate that the staircase method can track the time course of both analgesia and attenuation of this analgesia by an antagonist and can non-invasively monitor the time course of this activity with a resolution of one minute. The staircase method was also sensitive to the rate of infusion of a constant dose of fentanyl and the method may prove useful in the evaluation of putative opiate potentiators and related agents.

The Descriptor Differential Scale, formerly a paper-and-pencil clinical pain scale of moderate difficulty, has been adapted to a computer and is now presented in an easily used graphical format. This version of the scale will be validated in pain-free subjects and in those with dental postoperative pain and with pain from chronic conditions.

Assessment and Treatment of Chronic Pain

Our clinical trial studies evaluate mechanisms of pain and new treatment plans for neuropathy pain. Shingles pain is a neuropathy associated with sensory loss, hyperalgesia, allodynia and spontaneous pain. We are evaluating the effects of agents that increase the availability of serotonin or norepinephrine levels in the brain and thereby suppress the pain. This year we completed a study of the effects of desipramine in painful diabetic neuropathy and post-herpetic neuralgia. An interim analysis of the first 14 diabetic patients showed that pain ratings were lower with desipramine than with placebo from weeks 4 to 6 after drug administration. Sedation, dry mouth, and urinary slowing did not pose any problem for patients, in contrast to previous studies with amitriptyline. Amitriptyline is the standard treatment for pain related to nerve damage, but can be quite toxic to patients with medical illness or to elderly patients. Clinical trials with agents such as desipramine, with a narrower spectrum of action than amitriptyline, can offer an alternative treatment with fewer side effects and may also offer insight into the neurochemical mechanisms of tricyclic analgesia.

In another study we examined the effects of serotonin agonists in neuropathy pain. Twenty patients received two serotonin agonists, buspirone and mCPP, which act on $5HT_{1A}$ and $5HT_{1B}$ receptors, respectively. Little pain relief was reported with either serotonin agonist, at doses high enough to produce side effects in many of the patients receiving the drugs. Although these single dose studies did not reveal any effect of these agents, it is still possible that chronic use of such agents may be useful.

Mechanisms and Treatment of Acute Pain

We use an oral surgery model to evaluate novel agents, methods of administration, and drug combinations in comparison to standard analgesic therapy. The existence of multiple subtypes of opioid receptors which mediate both the analgesic and non-specific effects of opioids suggests that opioid analgesics which selectively bind to only one of these receptor subtypes may result in analgesia without the undesirable properties of traditional opioid analgesics. This hypothesis is being tested by evaluating the clinical efficacy and safety of a novel analgesic, spiradoline, which has been identified as a kappa receptor agonist. Fifty patients have participated in this study using the oral surgery model. Little activity was demonstrated for spiradoline in comparison to placebo when administered by gluteal injection. In contrast, the preliminary data indicate that the highest doses of spiradoline when administered by deltoid injection result in analgesic activity comparable to 10 mg of morphine. Further study is needed to confirm that spiradoline results in significant analgesia and to assess its side effect liability.

We have extended our examination of the role of bradykinin in inflammation by evaluating blood levels of bradykinin in patients undergoing oral surgery. As compared to preoperative levels, oral surgery resulted in a three-to-four fold elevation in circulating bradykinin. These findings indicate that bradykinin is synthesized and released during clinical inflammation in quantities sufficient to be detected in the vascular department. These findings suggest that pharmacological antagonists to bradykinin synthesis or receptor activity may have utility as analgesic and anti-inflammatory agents.

PUBLICATIONS

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

October 1, 1987 - September 30, 1988

Bennett GJ, Xie Y. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87-107.

Bennett GJ, Xie Y. An experimental peripheral neuropathy in rat that produces abnormal pain sensation. In: Dubner R, Gebhart GF, Bond MR, eds. Pain Research and Clinical Management; vol 3. New York: Elsevier, 1988;129-134.

Chudler EH, Pretel S, Kenshalo Jr, DR. Distribution of GAD immunoreactive neurons in the first (SI) and second (SII) somatosensory cortex of the monkey. Brain Research 1988;456:57-63.

Devor M, Dubner R. Centrifugal activity in C-fibers influences the spontaneous barrage generated in nerve-end neuromas. Brain Res 1988;446:396-400

Dionne RA. Peripheral pain mechanisms: Suppression of postsurgical dental pain with antiinflammatory analgesics. Anesth Prog 1987;34:113-127.

Dionne RA, Gift HC. Drugs used for parenteral sedation in dental practice. Anesth Prog 1988;35:198-204.

Dionne RA, Troullos ES. Pharmacologic control of acute pain. Clark's Clinical Dentistry Vol 1, 1988;1-10.

Dubner R. Research on pain mechanism in animals. JAVMA 1987;191:1273-1276.

Dubner R. Assessing pain in animals. Laboratory Animal Science, in press.

Dubner R. Commentary on clinical research: animal laboratory perspective. In: Chapman CR and Loeser JD, eds. Pain Measurement: Research and Clinical Issues. New York, Raven Press, in press.

Dubner R. The effect of behavioral state in the sensory processing of nociceptive and non-nociceptive information. Prog Brain Res, in press.

Dubner R. Effect of technical approaches on current opinions about the superficial dorsal horn. In: Cervero F, ed. Processing Sensory Information in the Superficial Dorsal Horn, in press.

Dubner R. Methods of assessing pain in animals. In, Textbook of Pain, edited by Melzack R and Wall PD, Churchill, in press.

Dubner R, Chudler EH, Kenshalo DR. Specialized pathways that subserve pain sensation. In: Takagi Y, Oomura N, Otsuka, eds. Naito Foundation International Symposium on Bio-warning System in the Brain, in press.

Dubner R, Gebhart GF, Bond MR. Proceedings of the Vth World Congress. Elsevier, Amsterdam, 1988;1-630.

Dubner, R., Hylden, J.L.K., Nahin, R.L. and Traub, R.J.: Neuronal plasticity in the superficial dorsal horn following peripheral tissue inflammation and nerve injury. In: Cervero F, ed. Processing of Sensory Information in the Superficial Dorsal Horn of the Spinal Cord, in press.

Dubner R, Sharav Y, Gracely RH, Price DD. Idiopathic trigeminal neuralgia: sensory features and pain mechanisms. Pain 1987;31:23-33.

Gracely RH. Verbal descriptor measures of pain clarify mechanisms of analgesia due to narcotics, brain stimulation and placebo. Anesthesia Progress 1987;34:113-127.

Gracely RH. Multiple random staircase assessment of thermal pain perception. In: Dubner R, Bond M, Gebhart G. eds. Proceedings of the Fifth World Congress on Pain. Amsterdam: Elsevier, 1988;391-394.

Gracely R. Methods of testing pain mechanisms in normal man. In: Wall PD, Melzack R. eds. Textbook of Pain. London: Churchill Livingstone, in press.

Gracely RH. Pain psychophysics. In: Chapman CR, Loeser JD, ed. Pain Measurement: Research and Clinical Issues. New York: Raven Press, in press.

Gracely RH, Kwilosz DM. The differential descriptor scale: applying psychophysical principles to clinical pain assessment. Pain, in press.

Gracely RH, Lota L, Walther DJ, Dubner R. A multiple random staircase method of psychophysical pain assessment. Pain 1988; 32:55-63.

Hargreaves K. Neuroendocrine modulation of pain. Anesth Prog 1987;34:121-23.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77-88.

Hargreaves K, Dubner R, Joris J. Peripheral action of opiates in the blockade of carrageenan-induced inflammation. In: Dubner R, Gebhart G, Bond M, eds. Pain research and clinical management, vol 3. Amsterdam: Elsevier Science Publishers, 1988;55-60.

Hargreaves K, Mueller G, Dubner R, Goldstein D, Dionne R. Corticotropin releasing factor (CRF) produces analgesia in humans and rats. Brain Res 1987;422:154-57.

Hargreaves K, Schmidt E, Mueller G, Dionne R. Dexamethasone alters plasma levels of beta-endorphin and post-operative pain. Clin Pharm Ther 1987;42:601-07.

Hargreaves K, Troullos E, Dionne R. Pharmacologic rationale for the treatment of acute pain. Dent. Clin of North Am 1987;31:675-94.

Hargreaves KM, Troullos ES, Dionne RA. Pharmacologic rationale for the treatment of acute pain. Dent Clin NA 1987;675-694.

Hargreaves K, Troullos E, Dionne R, Schmidt E, Schafer S, Joris J. Bradykinin is increased during acute and chronic inflammation: therapeutic implications. Clin Pharm Ther 1988 (in press).

Hylden JLK, Anton F, Nahin RL. Spinal lamina I projection neurons in the rat: collateral innervation of parabrachial area and thalamus. Neuroscience, in press.

Hylden, J.L.K., Nahin, R.L., Anton, F. and Dubner, R.: Characterization of lamina I projection neurons: physiology and anatomy. In: Cervero F, ed. Processing of Sensory Information in the Superficial Dorsal Horn of the Spinal Cord, in press.

Iadarola MJ, Brady LS, Draisci G, Dubner R. Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: stimulus specificity, behavioral parameters and opioid receptor binding. Pain, in press.

Iadarola MJ, Douglass J, Olivier C, Naranjo JR. Differential activation of spinal cord dynorphin and enkephalin neurons durng hyperalgesia: evidence using cDNA hybridization. Brain Res 1988;455:205-212.

Iadarola MJ, Flores CM, Cadet JL, Suddath R, Illowsky B, Berman KF. Met^5-enkephalin-arg^6-gly^7-leu^8 immunoreactivity in rat and human cerebrospinal fluid: influence of neuroleptic drugs and electroconvulsive shock. Brain Res 1988. In press.

Iadarola MJ, Flores CM, Yang H-Y. Release of [met^5]-enkephalin-arg^6-gly^7-leu^8 immunoreactivity into rat cerebrospinal fluid by electroconvulsive shock. Biochem Pharmacol 1987;36:801-802.

Iadarola MJ, Naranjo JR. Extraction of peptides from guanidine thiocyanate homogenates used to isolate RNA. Peptides 1988;9:669-671.

Iadarola MJ, Ruda MA, Cohen LV, Flores CM, Naranjo JR. In: Dubner R, Gebhart GF, Bond MR eds. Enhanced dynorphin gene expression in spinal cord dorsal horn neurons during peripheral inflammation: behavioral, neuropeptide, immunocytochemical and mRNA studies. Proceedings of the Vth World Congress on Pain. Amsterdam: Elsevier, 1988;61-71.

Joris J, Dubner R, Hargreaves K. Opioid analgesia at peripheral sites: a target for opioids released during stress and inflammation. Anesth and Analg 1987;66:1277-81.

Joris J, Reuter A, Vrindts-Gevaert Y, Gathy-Meuleman R, Hargreaves K, Franchimont P. Effect of diclofenac on plasma levels of immunoreactive prolactin, follicle stimulating hormone, luteinizing hormone, thyrotropin and beta-endorphin in humans. Hormone Res. 1988 (in press).

Kenshalo DR, Jr., Chudler EH, Anton F, Dubner R. SI neurons participate in the encoding process by which monkeys perceive the intensity of noxious thermal stimulation. Brain Res 1988;454:378-382.

Max MB, Gracely RH, Miser AW, Dionne RA, Hargreaves KH. Mechanisms of pain and analgesia. Anesth Prog 1987;34:113-127.

Max MB, Schafer SC, Culnane M, Dubner R, Gracely RH. Association of pain relief with drug side-effects in post-herpetic neuralgia: a single-dose study of clonidine, codeine, ibuprofen, and placebo. Clin Pharmacol Ther 1988;363-371.

Max MB, Schafer SC, Culnane M, Smoller B, Dubner R, Gracely RH. Amitriptyline, but not lorazepam, relieves post-herpetic neuralgia. Neurology 1988, in press.

Miser AW, Moore L, Greene R, Gracely RH, Miser JS. Prospective study of continuous intravenous and subcutaneous morphine infusions for therapy-related young adults with cancer. Clin J Pain, in press.

Nahin RL. Immunocytochemical identification of long ascending peptidergic neurons contributing to the spinoreticular tract in the rat. J. Neurosci 1987;23:859-869.

Nahin RL. Immunocytochemical identification of long, ascending peptidergic lumbar spinal neurons terminating in either the medial or lateral thalamus in the rat. Brain Res 1988;443:345-349.

Pretel S, Ruda MA. A comparison of noradrenaline, substance P and enkephalin axonal input to serotonin neurons in the caudal raphe nuclei of the cat. Neuroscience Letters 1988;89:19-24.

Rothman RB, Danks JA, Iadarola MJ. Unexpectedly high levels of opioid peptides in rat brain membranes. Peptides 1987;8:645-649.

Ruda MA. Spinal dorsal horn circuitry involved in the brainstem control of nociception. Progress in Brain Res, in press.

Ruda MA. Endogenous opioid response to peripheral nerve injury and arthritis. In: Proceedings of NIH Workshop on New Directions in Lowback Pain Research, in press.

Ruda MA, Allen B, Cohen L, Humphrey E, Iadarola M, Shiosaka S, Takahashi O, Young WS III. *In situ* hybridization histochemical and immunocytochemical analysis of opioid gene products in a rat model of peripheral inflammation. In: Proceedings of NATO Advanced Research Workshop, in press.

Ruda MA, Iadarola M, Cohen L, Young WS III. *In situ* hybridization histochemistry and immunocytochemistry reveal an increase in spinal dynorphin biosynthesis in a rat model of peripheral inflammation and hyperalgesia. Proc Nat Acad Sci 1988;85:622-626.

Sharav Y, Singer E, Schmidt E, Dionne RA, Dubner R. The analgesic effect of amitriptyline on chronic facial pain. Pain 1987;31:199-209.

Tashiro T, Ruda MA. Immunocytochemical identification of axons containing coexistent serotonin and substance P in the cat lumbar spinal cord. Peptides 1988;9:383-391.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00031-20 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Design and Computer Interfacing of Neurophysiologic Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Frederick J.	Electronic Engineer (Instru)	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	----

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects involve the design and construction of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory and central computer installations. Electronic circuit design, microcomputers, and assembly or machine language programming may be used in these instruments or interfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00132-14 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacological Modification of Neuroendocrine Responses to Surgical Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hargreaves, Kenneth	Staff Fellow	NA NIDR
Dionne, Raymond	Research Pharmacologist	NA NIDR
Costello, Ann	Post Doctoral Fellow	NA NIDR
Troullos, Emanuel	Post Doctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

Jackson, William	Medical Staff Fellow	LOM NIDR
Schmidt, Elizabeth	Clinical Nurse	CC Nursing
Schafer, Susan	Clinical Nurse	CC Nursing

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.05	1.90	.15

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of two related lines of research evaluating 1) the neuroendocrine responses to surgical stress and inflammation, as evidenced by measuring changes in immunoreactive bradykinin (iBK) and beta-endorphin (iB-END), and 2) the analgesic and anti-inflammatory effects of prototype and novel analgesic drugs on altering either the synthesis or the receptor activation of these mediators. Evidence supporting bradykinin's involvement in clinical models of inflammation comes from our finding that blood-borne levels of immunoreactive bradykinin (iBK) are significantly elevated during the surgical removal of impacted third molars. This finding extends last years observations that iBK is significantly elevated in patients with rheumatoid arthritis and in rats with carrageenan-induced inflammation. The potential utility of bradykinin antagonists were evaluated in rats using drugs which either block the synthesis of iBK or which act as receptor antagonists. Prototype drugs representing both pharmacological approaches displayed significant anti-inflammatory activity in the rat carrageenan model of inflammation. This year, we have also continued our research on the pituitary secretion of immunoreactive beta endorphin (iB-END) in response to inflammation. Our results indicate that inflamed tissue from both rat and human samples, contains a peripheral corticotroph-stimulating factor which promotes iB-END release both in cultured pituitary cells and in vivo. Thus, the pituitary-adrenal response to a stressor may be a result of both hypothalamic (i.e., release of CRF) and extra-hypothalamic input, with stressors differing in their activation of these two systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00133-14 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	.80	1.45

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement; (2) to assess clinical pain measures in a dental setting; (3) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses; and (4) to evaluate known pharmacological and non-pharmacological pain-control agents. The interactive computer-based staircase scaling method was used in five experiments.

The first experiment, performed in dental patients immediately before an oral surgical procedure, showed that the staircase method successfully tracks the time course of both the analgesia produced by narcotic infusion and the reversal of this analgesia by administration of a narcotic antagonist. The method also showed hyperalgesia following antagonist administration in comparison to placebo, suggesting sensitivity to endogenous opioids released in anticipation of oral surgery.

The second experiment assessed time course of 1.1 ug/kg fentanyl analgesia following infusion rates of 2, 5, 10 and 20 min to evaluate the model's potential for detecting opioid analgesia enhancement by potentiating agents. Analgesia was rate dependent and rates of 10-20 mins appear ideal for agonist evaluations.

The third experiment assessed the influence of the serotonergic agonist MCPP on pain sensitivity in pain-free subjects. MCPP had no apparent effect, a result consistent with another study in this laboratory in which MCPP did reduce pain associated with postherpetic neuralgia.

The fourth experiment investigated if the absence of cardiac chest pain during "silent ishcemia" may represent a natural analgesic state detectable by experimental methods. Preliminary results suggest that patients with silent ischemia show normal sensitivity to thermal pain sensations.

The fifth study assessed if two weeks treatment with the narcotic antagonist naltrexone increases opiate sensitivity. The results of three initial cases show no effect which may represent premature assessment after naltrexone termination resulting in residual opiate antagonism.

A new computerized version of the DDS scale was tested in pain-free subjects. The method is used more easily and provides increased range and less bias effects than the previous paper and pencil version.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00246-11 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Pharmacological Management of Chronic Orofacial Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond	Research Pharmacologist	NA NIDR

COOPERATING UNITS *(if any)*

Schmidt, Elizabeth	Nurse	CC Nursing
Lipton, James	Chief, PEC	OPEC NIDR

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is attempting to evaluate pharmacological approaches to the clinical management of chronic facial pain and to develop better methods for its differential diagnosis. Two clinical trials have evaluated standard pharmacological agents for the management of two different variants of chronic facial pain: myofascial pain dysfunction (MPD) and atypical facial pain (AFP). These studies seek to evaluate the efficacy of prototypic pharmacologic regimens under conditions of a controlled clinical trial.

Thirty-nine subjects completed the MPD study. Pain relief, as measured by the pain relief VAS, revealed significantly greater relief from diazepam than ibuprofen or placebo. No significant changes were seen in depression or anxiety in comparison to baseline or between groups. These data indicate that diazepam results in symptomatic relief of MPD when evaluated under conditions of a controlled trial, suggesting that anxiolytic drug therapy is effective for the relief of myofascial pain while NSAIDS have minimal therapeutic benefit when used alone.

Amitriptyline was more effective than placebo in reducing atypical facial pain after four weeks of treatment. When patients were divided into depressed and non-depressed groups, based on their Hamilton depression scores, amitriptyline reduced pain in the depressed and in the non-depressed groups as compared to placebo. Amitriptyline reduced the depression scores in the depressed group but had no effect on the depression scores in the non-depressed group. These data suggest that amitriptyline is effective in the treatment of chronic oral-facial pain and that its efficacy is independent of its effects on depression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-10 NA

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Stimulation Analgesia in the Control of Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Dionne, Raymond A.	Research Pharmacoloigst	NA NIDR
Max, Mitchell B.	Neurologist	NA NIDR

COOPERATING UNITS *(if any)*

Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.90	.65	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in non-surgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimental induced pain will be assessed in pain-free volunteers.

A follow-up of one patient supports previous findings from this project. This patient, who received an implantable stimulator in April 1986, reported moderate pain relief in a postoperative visit to the NIH in May 1986. He presently attributes minimal pain relief to his stimulator, and provides evidence that his previously reported relief may reflect an ambitious exercise program and not the effects of stimulation. These findings are consistent with previous results of this project. Analgesia from deep brain stimulation is not opioid mediated, is less potent than morphine analgesia and follows a longer time course than analgesia following narcotic administrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00286-09 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Therapeutics for Acute Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond	Research Pharmacologist	NA NIDR
Costello, Ann	Postdoctoral Fellow	NA NIDR
Troullos, Emmanuel	Postdoctoral Fellow	NA NIDR
Carson, Daniel	Postdoctoral Fellow	NA NIDR
Hargreaves, Kenneth	Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*

Schmidt, Elizabeth	Nurse	CC Nursing
Samaha, Ramona	Nurse	CC Nursing

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	2.55	.70

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. A recently completed study evaluated the interaction of proglumide, an antagonist of cholecystokinin, with morphine for postoperative pain. Current studies are evaluating SCH 34826, a novel agent which inhibits the breakdown of enkephalin in the central nervous system, and spiradoline, a novel analgesic which acts at the kappa opioid receptor. The surgical removal of impacted third molars serves as a model for minor surgical procedures with associated intraoperative and postoperative pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety.

Administration of 0.05 mg of proglumide was demonstrated to significantly potentiate and prolong the analgesic efficacy of 4 mg of proglumide for postoperative pain while higher doses of proglumide were without effect. These data provide support for the use of proglumide for the potentiation of opioid analgesia but without the side effects associated with higher doses of an opioid. Preliminary results of the evaluation of spiradoline failed to demonstrate any significant analgesia following intramuscular administration in the gluteus. IM administration in the deltoid resulted in detectable analgesia and provides the rationale for continued study of this drug as a selective opioid analgesic. The evaluation of the enkephalinase inhibitor, SCH 34826, has not been completed.

Demonstration of significant potentiation of morphine analgesia by proglumide may provide a rational basis for the pharmacologic management of pain without the limitations of higher doses of opioids. The preliminary results suggesting that spiradoline results in clinical analgesia provide a basis for continued investigation of experimental agents which act selectively through opioid receptor sub-types to determine if greater selectivity of analgesic action can be achieved. These strategies and the continuing evaluation of the enkephalinase inhibitor may provide support for therapeutic alternatives to traditional analgesic therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00288-09 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropharmacological Characterization of Synaptic Circuitry in Dorsal Horn

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ruda, Maryann	Research Biologist	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Humphrey, Emma L.	Biol. Lab. Tech. (Elec. Mic.)	NA NIDR
Takahashi, Osamu	Fogarty Visiting Scientist	NA NIDR
Solodkin, Ana	Guest Researcher	NA NIDR
Traub, Richard	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.00	3.90	2.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The neural circuitry of the dorsal horn of the spinal cord forms the basis for the mechanisms of pain and neuralgia. Our lab has made significant inroads in understanding the neuronal connectivity which subserves these sensory phenomena through experiments involving multiple markers to identify interactions between neural elements.

In situ hybridization histochemistry was used to examine changes in opioid gene expression in a model of peripheral inflammation and hyperalgesia. No detectable change in preproenkephalin mRNA was visible in spinal dorsal horn neurons at the same time that preprodynorphin mRNA exhibited a dramatic increase. These observations suggest that the two opioid systems are responding differentially to prolonged nociceptive inputs.

Afferent input to spinal dynorphin neurons which exhibit an upregulation during peripheral inflammation and hyperalgesia were examined using double label immunocytochemical methodology. Calcitonin gene-related peptide containing primary afferent axons were observed to contact dynorphin neurons in laminae I, II and V. These contacts may provide a direct link between peripheral nociceptive stimuli and opioid neurons in the dorsal horn.

The rostral projection of small diameter primary afferents was examined in the lumbar spinal cord. Immunocytochemical staining for calcitonin gene-related peptide demonstrated that labeled afferents in Lissauer's tract and laminae I, II and V traveled at least five segments from their level of entry into the spinal cord. Within the spinal cord, these afferents are unmyelinated. These observations provide new evidence on the extent of small diameter primary afferent projections within the spinal cord.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 DE 00291-09 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Medullary Dorsal Horn of the Behaving Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David A.	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.85	.30	.55

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We examined the effects of ST-91 microinjected into the medullary dorsal horn (MDH) on the ability of monkeys to detect small temperature increases in the noxious heat range. Behavioral detection latency and the percentage of correct detections were used as measures of the monkeys perceived intensity of noxious thermal stimulation. The monkey was trained to detect temperature changes of 0.4, 0.6, and 1.0°C superimposed on an initial temperature shift of 46°C (T1). ST-91, an α-2 adrenergic agonist, was microinjected (1,3,10 micrograms) into the MDH and produced dose-dependent and stimulus-dependent increases in the detection latency to T2 noxious heat stimuli. there was no effect of ST-91 on the innocuous cooling or light trials indicating that the changes were modality specific and independent of motivation, attention and motoric factors. These data demonstrate a pharmacologically-specific effect of an α-2 adrenergic agonist on the perceived intensity of noxious heat stimuli at the earliest central relay for noxious information.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00329-07 NA

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Discrimination of Thermal Stimuli Applied to the Face in Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dubner, Ronald	Chief, NAB	NA NIDR
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanism Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.30	.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project correlates behavioral responses with neural responses of thalamic projection and non-projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) produced by noxious thermal stimuli in the behaving monkey. Medullary dorsal horn neurons encode thermal discriminative information used by the monkey to perform a thermal detection task. Many medullary dorsal horn neurons encode thermal intensity in a manner which allows the detection of small changes in noxious temperatures. The role of dorsal horn wide-dynamic-range (WDR) and nociceptive-specific (NS) neurons in the encoding of the perceived intensity of noxious stimuli was determined while monkeys detected near-threshold changes in the intensity of noxious heat stimuli. Behavioral detection latencies were a reliable measure of the perceived intensity of these stimuli. There was a significant correlation between behavioral detection latency and neuronal discharge of WDR, but not NS neurons. In addition, WDR neurons exhibited greater activity on correctly-detected versus non-detected trials, whereas NS neurons did not. We conclude that WDR neurons are involved in the encoding process by which monkeys perceive the intensity of noxious heat stimuli near detection threshold. Some thermally sensitive neurons also respond to other stimuli used by the monkey for the successful completion of the task. This task-related activity occurs in characteristic patterns of excitation and/or inhibition and some neurons which exhibit such activity project to the thalamus. The task-related responses exhibited by some of these neurons may modulate sensory activity and thereby influence the perception of and response to oral-facial pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-06 NA

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Max, Mitchell	Neurologist	NA NIDR
Kumar, Ranganna Kishore	Medical Staff Fellow	NA NIDR
Lynch, Sue	Medical Staff Fellow	NA NIDR
Zeigler, Daryl	Visiting Fellow	NA NIDR
Gracely, Richard	Research Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

Schafer, Susan	Nurse	CC Nursing
Muir-Nash, Joanne	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	2.20	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to elucidate the principles of treatment of chronic pain syndromes that are considered resistant to conventional treatment, such as pain caused by peripheral neuropathy and nerve injury, and by advanced cancer.

In a randomized, double-blind, crossover study, 20 patients each with diabetic neuropathy and post-herpetic neuralgia were treated with 6 weeks each of placebo and desipramine, an antidepressant that selectively blocks synaptic reuptake of norepinephrine. An interim analysis of the first 14 diabetics who completed the study showed that desipramine was superior to placebo in relieving pain, and caused fewer side-effects than had been observed in previous studies with amitriptyline, the standard medication. In another study, 20 patients with neuropathic pain showed no analgesic response to single doses of two serotonin agonists, buspirone and mCPP. These results support the hypothesis that the potentiation of noradrenergic neural activity is sufficient to inhibit some neuropathic pain states, and that acute stimulation of serotonergic receptors may not be sufficient for analgesia. To directly examine these questions, a comparison of amitriptyline, desipramine, and fluoxetine (a specific blocker of serotonin reuptake) is now beginning.

Several new research programs were initiated and patients accrued: A collaborative program with NCI investigating the potentiation of opiate analgesia by the CCK antagonist proglumide and by desipramine; a post-operative pain program with the Bethesda Naval Hospital Department of Surgery studying the effects of pre-operative treatment with prostaglandin inhibitors on post-operative pain; and a program of detailed sensory testing to elucidate the mechanism of neuropathic pain states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00377-05 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of the Primate Primary Somatosensory Cortex in Nociception

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR
Chudler, Eric H.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David Alan	Psychologist	NA NIDR
Iwata, Koichi	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.55	1.60	.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies is to examine the role of the primary somatosensory cortex (SI) in nociception. We examined the response of nociceptive neurons in SI to small temperature changes superimposed on noxious levels of thermal stimulation. Two populations of nociceptive neurons were found: one population encoded the intensity of noxious thermal stimulation; the other responded to noxious thermal stimulation, but did not encode the intensity.

While major differences in the response of SI nociceptive neurons were found to large temperature changes, no differences were found in the two populations response to small temperature changes. The neuronal discharge of SI nociceptive neurons was correlated with the monkeys' ability to detect noxious thermal stimulation. A significant correlation was found between the peak neuronal discharge and the monkeys' detection speed. In addition, the discharge of SI nociceptive neurons was significantly greater on correctly detected vs non-detected trials. Increases in foreperiod produced an increase both in the monkeys' detection speed as well as an increase in the neuronal discharge of SI nociceptive neurons. These results put to rest the long held view that the cerebral cortex does not participate in the encoding of stimulus features in the noxious range. We conclude that SI cortical nociceptive neurons are involved in the process by which monkeys perceive the intensity of noxious thermal stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 DE 00413-03 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Neuropathy of Peripheral Nerve in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Research Biologist	NA NIDR
Kajander, Keith C.	Postdoctoral Fellow	NA NIDR
Wakisaka, Satoshi	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.25	3.00	.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A unilateral experimental neuropathy is produced in the sciatic nerve of rats. The postoperative behavior of these animals indicates that the nerve injury produces disordered pain sensations like those that are seen in humans with nerve injuries due to trauma or disease. The symptoms of neuropathic pain in these animals include hyperalgesia, allodynia, and spontaneous pain. We have shown that the nerve injury evokes large changes in the neurochemistry of small diameter, primary afferent neurons whose axons travel in the injured nerve. The changes begin within 5-10 days and persist for 2-3 months. The changes seen to date are decreases in the levels of substance P, calcitonin gene-related peptide and fluoride-resistant acid phosphatase. The decreases have been seen in the neurons' somata in the dorsal root ganglia and also in their terminal arbors in spinal dorsal horn laminae I-II and V. An additional change has been noted in intrinsic spinal neurons. Within days of the nerve injury, there is a large increase in spinal levels of the opioid peptide, dynorphin. This increase is initially found in neurons in laminae I and V-VI. By 10 days, however, only the deeper cells can be detected. Examination of toluidine blue-stained thin sections has shown that many small neurons in laminae I-III have the increased cytoplasmic and nucleo-plasmic staining density that precedes neuronal degeneration. The symptoms of disordered pain sensation are detectable 2 days after the nerve injury. Nerve function has been studied during the prodromal period (1 day old injuries) and we have found marked abnormalities. A very large majority of the nerve's myelinated axons cannot conduct impulses across the injured region, but nearly all of the unmyelinated fibers conduct normally. About 25% of the myelinated fibers emit spontaneous, ectopic discharges that are exacerbated by gallamine triehiodide. It is known from other experiments that the gallamine effect reflects an abnormality in voltage-sensitive potassium conductance and this, in turn, is a likely mechanism for the production of the spontaneous discharge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-03 NA

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CNS Neurotransmitter Regulation During Peripheral Inflammatory States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Iadarola, Michael	Senior Staff Fellow	NA NIDR
Draisci, Gaetano	Guest Researcher	NA NIDR
Yeung, Choh	Biologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.95	2.00	.95

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the role of CNS peptide-containing neurons in sensory processes especially as they relate to pain and the control of pain. A model of peripheral inflammation has been developed to investigate the relationship between spinal cord opioid-containing neurons (enkephalin and dynorphin) and abnormal primary afferent input. Alterations in opioid gene expression are assessed by measurement of peptide and mRNA levels and in situ hybridization techniques. Molecular mechanisms regulating dynorphin gene transcription are presently being investigated.

The time course and stimulus requirements for the increase in spinal cord dynorphin gene expression have been fully characterized. The earliest time reliable changes in preprodynorphin mRNA levels can be measured is 4 hours after inflammation. This now permits us to perform pharmacological experiments on induction and blockade of the response. The inflammation also induces an increase in c-fos proto-oncogene mRNA that is very rapid in onset (within 30 min). These results suggest that c-fos, a nuclear protein, may be involved in transcriptional regulation of the dynorphin gene. A new technique for localizing DNA binding proteins and possible transcription factors has been employed to identify additional proteins that bind to the 5-prime end of the dynorphin gene. It is in this region that most transcriptional regulation occurs. Evidence for a protein specific to neural tissue and certain neural-derived cell lines has been obtained.

The significance of these studies is that they reveal which opioid neurons in spinal cord are activated in response to inflammatory pain and, possibly, pain associated with arthritis and cancer. Further elucidation of the pivital role of the spinal dynorphin system in nociceptive processes and mechanisms underlying the increase in gene expression may provide new avenues for the pharmacotherapy of pain and new insights into chronic opioid abuse and tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00440-02 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dorsal Horn Circuitry Related to Pain and Hyperalgesia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hylden, Janice L.K.	Staff Fellow	NA NIDR
Nahin, Richard L.	Staff Fellow	NA NIDR
Traub, Richard J.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiolgy Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.75	PROFESSIONAL: 1.50	OTHER: .25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the present research project, we have employed a combination of physiological, pharmacological and behavioral approaches to the study of somatosensory systems related to pain and hyperalgesia.

The activity of lumbar spinal dorsal horn lamina I projection neurons was studied in normal rats and in rats with an inflamed hindpaw. Inflammation was produced by injecting Freund's adjuvant into the plantar surface 4 hours to 5 days prior to electrophysiological recording. Neurons recorded ipsilateral to the inflammation had enlarged and more complex receptive fields as compared to those observed in control rats. Many cells had high background firing rates including some burst activity and some cells responded to joint movement in addition to cutaneous stimulation. The increased activity of this population of neurons may result in a range of pain sensation. We have also begun to examine the central changes that occur during inflammation by characterizing the spinal cord pharmacology of nociception/antinoception in a behavioral model of hyperalgesia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DE 00459-01 NA

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Distribution of CABAergic Neurons in the Somatosensory Cortex of the Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Chudler, Eric H.	Postdoctoral Fellow	NA NIDR
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR
Nahin, Richard L.	Staff Fellow	NA NIDR
Pretel, Stephanie	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neural Mechanisms Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.95	.80	.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The distribution of neurons immunoreactive for gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase (GAD) was examined in four regions of the primate cortex that receive somatosensory input. In the first study, the distribution of neurons immunoreactive for GAD, the synthesizing enzyme of GABA, was examined in the first (SI) and second (SII) somatosensory cortex. GAD-like immunoreactive (GAD-LI) somata and puncta were present in all layers of SI and SII and were classified as nonpyramidal neurons. GAD-LI somata were most numerous in layer IV of SI and in layer III of SII. Additionally, a dense band of GAD-LI punta was present in layer IV of SI, but was not observed in any layer of SII. The major diameters of GAD-LI somata in SII were significantly smaller than those in SI in layers I, II and IV.

In the second study, the distribution of neurons immunoreactive for GABA, a major inhibitory neurotransmitter, was examined in the retroinsular cortex (Ri) and area 7b of the monkey. As with GAD-LI neurons in SI and SII, GABA-like immunoreactive (GABA-LI) somata in Ri and area 7b were observed in all layers. However, the majority of somata in Ri and area 7b were observed in the superficial cortical layers (I and II). Moreover, in contrast to the high numbers of large (greater than 15 um) labelled somata in SI and SII, less than 1% of all the somata in Ri and area 7b had major diameters greater than 15 um.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00460-01 NA

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neural Circuitry of the Dorsal Horn in Normal and Hyperalgesic Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nahin, Richard L.	Staff Fellow	NA NIDR
Hylden, Janice L.K.	Staff Fellow	NA NIDR
Dubner, R.	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the present research, we have employed a variety of anatomical techniques to study the neural circuitry of the spinal cord as it relates to pain and hyperalgesia.

Lamina I neurons were retrogradely labeled after injections into the caudal midbrain of animals with inflamed hindpaws. Spinal cord sections from these animals were processed for dynorphin immunoreactivity. It was found that the number of lamina I projection neurons immunoreactive for dynorphin was substantially greater in spinal cord sections ipsilateral to the inflamed hindpaw.

Spinal cord section of normal animals were processed for GABA immunoreactivity and either Calcitonin Gene Related Peptide (CGRP) or Enkephalin (ENK) immunoreactivity. It was found that a small number of GABAergic neurons were contacted by either CGRP or ENK varicosities. On the other hand, large numbers of GABA varicosities were in apposition to ENK-containing neurons.

ANNUAL REPORT OF THE LABORATORY OF ORAL MEDICINE
NATIONAL INSTITUTE OF DENTAL RESEARCH

The emphasis in the Laboratory of Oral Medicine over the last year has been on: (I) autoantibodies; (II) autoantigens; (III) insulin-dependent diabetes mellitus; (IV) herpes simplex virus; and, (V) human immunodeficiency virus (HIV) and transgenic mice. A major effort has been put into HIV and the development of a mouse model using transgenic technology.

The Laboratory is involved in a number of collaborative projects including: (1) preparation of human monoclonal antibodies (Cetus Immune Research Laboratories, Palo Alto); (2) studies related to EBV (Scripps Clinic and Research Foundation, La Jolla; DCT and DBB, NCI and FDA); (3) studies on human V_H and V_L genes (University of Texas, South Western Medical School, Dallas and Scripps Clinic and Research Foundation, La Jolla); (4) human monoclonal antibodies to HIV and rabies virus (LTLB, NCI and LIR, NIAID, and The Wistar Institute, Philadelphia); (5) HLA DR expression in Sjogren's Syndrome (CIPCB, NIDR); (6) RFLP of thyroid autoantigen and TSH binding to lymphocytes (DCBD, NCI and LBM, NIDDK); (7) cloning and identification of thyroid autoantigens and TSH receptor (LBP, NIDDK); (8) genomic mapping of the genes for human thyroid autoantigens (DCBD, NCI); (9) RFLP of HLA in autoimmune diseases (ARB, NIAMS); (10) molecular basis of antibody specificity (LG, NCI); (11) virus-induced myocarditis (University of Nebraska Medical Center, Omaha, and Rockefeller University, New York); (12) tests for islet cell autoantibodies in diabetes using two dimensional Western blots (LCS, NIMH); (13) identification of diabetic autoantigens using patients' sera (Mount Sinai School of Medicine, New York); (14) POMC gene expression in lymphocytes (IR, NIAID); (15) cloning of islet cell autoantigens (University of Washington, Seattle); (16) factors that trigger and inhibit reactivation of HSV (LCI, NIAID); (17) vaccinia/HSV recombinant vaccine for HSV (LVD and LID, NIAID); (18) topical indomethacin as blocking agent for reactivation of HSV (UCLA School of Medicine, Los Angeles); (19) epithelial irritants as triggers of HSV reactivation (UCLA School of Medicine, Los Angeles); (20) transgenic mice carrying the genome of HIV (LMM, NIAID); (21) production of transgenic mice carrying a gene for early protein of human melanoma (The Wistar Institute, Philadelphia); (22) detection and cloning of oncogenes from human endocrine tumors (LI, NCI); and (23) cloning and characterization of thyroid autoantigens (LI, NCI).

Since last year, a number of new techniques were introduced into the Laboratory and existing ones modified. These include: (1) polymerase chain reaction to amplify Epstein-Barr viral DNA in biopsy material from patients with rheumatoid arthritis; (2) preparation of synthetic peptides from deduced amino acid sequences of novel thyroid autoantigen recently cloned in our Laboratory; (3) synthesis of oligonucleotides for sequencing and polymerase chain reactions; (4) RNA extraction from mouse tissues and Northern analysis to study the expression of transgene; (5) pGEM72 vector and Erase-a-Base System for rapid construction of plasmids for efficient sequencing of large DNA fragments; (6) Baculovirus plaque hybridization for selecting recombinants that contain the cDNA of interest; (7) expression of novel autoantigen using the Baculovirus expression system; (8) human chromosome specific λ phage libraries for screening of clones containing specific sequences; (9) subcloning of genomic fragments into plasmids for genomic "walking"; (10) bi-directional DNA dideoxy sequencing of the genomic DNA; (11) improved methods for producing stable cell lines which secrete human monoclonal autoantibodies; (12) characterization of $\underline{V}_H$ gene segments in human antibody-secreting monoclonal cell lines; (13) cosmid cloning using the vector pWE15 to test for expression of cloned genomic DNA in eucaryotic cells; (14) hexagonal array pulse field electrophoresis to separate high molecular weight DNA with better resolution.

Some of the more important findings since last year's annual report are summarized below:

I. AUTOANTIBODIES AND THEIR PROPERTIES

1. $\underline{\text{CD5}^+}$ B Lymphocytes Make Rheumatoid Factor: Last year, we showed that polyreactive antibodies were made by CD5^+ B lymphocytes. This year, we found that the majority of CD5^+ B cells from healthy subjects are committed to the production of antibodies with rheumatoid factor activity. By fusing the EBV-transformed cells generated from CD5^+ B lymphocytes with human-mouse heterohybrid cells, we constructed continuous cell lines producing monoclonal antibodies (mAb). These mAb bound not only to the Fc fragment of IgG, but also at varying degrees to other self-antigens, such as ssDNA, thyroglobulin and insulin, as well as to exogenous antigens, such as tetanus toxoid, LPS from Gram-negative and polysaccharides from Gram-positive bacteria. Competitive inhibition studies revealed that although each of the mAb reacted with the Fc fragment of IgG, their functional affinities for other Ag varied by as much as 1000-fold. Our studies argue that broad polyreactivity is an inherent property of the antibodies produced by CD5^+ B lymphocytes and that these antibodies may be what has been referred to as the "natural antibodies" of the serum.

2. Analysis of the Human B Cell Repertoire: Using EBV as B cell activator and limiting dilution analysis, we are now able to analyze and quantitate human peripheral blood B lymphocytes capable of producing antibodies to self and exogenous molecules. We found that B cells capable of producing IgM, IgG, and IgA antibodies to thyroglobulin (Tg), insulin (Ins), IgG Fc fragment and thyroid mucosomal antigens are common components of B-cell repertoire in healthy subjects. Moreover, we found that many of the monoclonal antibodies generated from healthy subjects are polyreactive, that is, they bind to a number of different antigens. In the condition of enhanced autoimmune response, such as Hashimoto's disease, insulin-dependent diabetes mellitus (IDDM) and systemic lupus erythematosus (SLE), the number of B cells capable of producing IgG to Tg, Ins and ssDNA, respectively, is dramatically increased. However, Hashimoto's, IDDM or SLE patients do not differ from healthy donors in the number of B cells producing IgM or IgA to

irrelevant autoantigens. Moreover, many of the monoclonal autoantibodies generated from autoimmune diseases were monoreactive for the relevant autoantigens. In contrast, monoclonal autoantibodies generated from healthy subjects were most often polyreactive. This approach for quantitating the B cell repertoire is providing new insights into the pathogenesis of autoimmune disease.

3. Model For Studying Virus Attachment: Epstein-Barr virus (EBV) was purified and biotinylated without significant loss of its cell-transforming activity. The use of biotinylated virus in conjunction with antibodies specific for selected cell surface molecules and flow cytometric analysis allowed for the positive identification of the virus-binding lymphocytes among a heterogeneous mononuclear cell population. Biotinylated EBV efficiently bound to all B lymphocytes, including those bearing surface μ, δ, γ and α immunoglobulin heavy chains, or the surface CD5 (Leu-1) marker, but not to T lymphocytes, natural killer cells, or monocytes. By using biotinylated EBV and specific monoclonal antibodies in competitive inhibition experiments, it also was found that the virus attaches to an epitope on the CR2 molecule (the receptor for C3d and EBV), which is close to or identical with the one recognized by OKB7 monoclonal antibody, and that cell surface structures other than CR2 cannot mediate attachment of EBV. Moreoever, studies on the binding of the virus to induced B lymphocytes at different stages of the cell cycle revealed that the virus failed to transform proliferating B lymphocytes (cells in S through G_2 phase), and this was associated with the disappearance of the surface CR2 molecule and the inability of the virus to attach to these cells. The approach should be useful in studying the attachment of other viruses, identifying the specific cell types involved, and analyzing the effect of the cell cycle on virus binding. In fact, studies in progress indicate that this approach is useful for studying the binding and identifying the cell type to which human retroviruses attach.

II. AUTOANTIGENS

1. Cloning and Sequencing Autoantigens: Patients with Graves' disease have circulating antibodies directed at thyroid antigens, including the thyroid stimulating hormone (TSH) receptor. The molecular identity of a number of these autoantigens remains unknown. The present study, in collaboration with Dr. Len Kohn of NIDDK, was initiated to isolate clones that code for some of these unidentified autoantigens. The general approach was to isolate cDNA clones by screening a human thyroid carcinoma λgt11 library with immunoglobulins purified from serum of a patient with autoimmune Graves' disease. One clone (ML8) containing a 1.25kb insert hybridized with a single 2.0kb poly A+ mRNA in human thyroid

and lymphocytes, but not in human brain, liver, kidney, or muscle. In addition, this probe also hybridized with a single 2.0kb poly A^+ mRNA from a rat thyroid cell line (FRTL-5). An apparently full-length 2,074bp human cDNA was obtained and sequenced. The nucleotide sequence of the 2,074bp cDNA includes a 5′ non-coding sequence of 17bp, a 1827bp open reading frame, and a 222bp 3′ non-coding sequence. The canonical poly-adenylation signal AATAAA is present 18bp upstream of the poly A tail. This cDNA encodes a 69,812 dalton protein with two potential N-linked glycosylation sites and at least one potential membrane spanning domain. Immunoprecipitation of the in vitro translated protein by sera from several patients with Graves' disease argues that the 69,812 dalton is an autoantigen that may be involved in Graves' disease.

2. Expressing Autoantigens: The Baculovirus expression system was used to express the 2.0kb cDNA. The Baculovirus system was chosen because of the potential to produce large amounts of a glycosylated protein. A 2.0kb cDNA was inserted into the Baculovirus genome under the control of the strong polyhedrin promoter. Insect cells infected with the recombinant virus express a 70kD protein in large amounts. This protein does not appear to be secreted. Oligopeptides were synthesized, based on the cDNA sequence, and antibodies to these peptides bound to the 70kD protein on Western blots. Detailed studies are in progress to purify and characterize this protein. The Baculovirus-expressed protein may be a useful tool for screening patient sera in the diagnosis of Graves' disease. The ultimate goal of this and related research on IDDM is the early detection of the onset of autoimmune processes that might lead to the prevention of these diseases.

III. INSULIN-DEPENDENT DIABETES MELLITUS

1. Oncogenes From Endocrine Tumors: Gene transfer techniques have revealed the presence of oncogenes in many tumors and tumor cell lines. Most of these genes belong to the ras family. Methods were introduced into our Laboratory last year to allow the detection and cloning of both strong and weakly transforming oncogenes from a given tumor. Tumor DNA was co-transfected with a selectable marker gene (neo) into NIH/3T3 cells and the resulting drug resistant colonies were divided into 10 pools for tumorigenesis testing in athymic nude mice. Since each pool represents less than one genome of transfected tumor DNA, this approach might allow the separation and detection of more than one oncogene from each original tumor. Using the sib-selection approach, we have isolated an activated N-ras gene from a lymph node metastasis of a highly invasive renal cell carcinoma. We are currently sequencing the gene to determine the site of mutation and plan to use a specific oligonucleotide probe to determine if this mutation is also found in the original tumor and other metastases in the same patient. We also plan to

survey polymerase chain reaction amplified DNA from other renal cell carcinomas to determine the prevalence of this mutation in this type of cancer. The sib-selection procedure was also used to detect a non-ras related oncogene from a human insulinoma (tumor of beta cell origin). This weakly transforming oncogene induces tumors in nude mice approximately 10 weeks after injection. The insulinoma associated oncogene is currently being cloned for further analysis. The sib-selection tumorigenesis assay may facilitate the discovery of new oncogenes associated with other slow growing differentiated endocrine malignancies.

2. Ten-Year Longitudinal Study on Children at High Risk of Ultimately Developing Insulin-Dependent Diabetes Mellitus (IDDM): The frequency of IDDM in the general population is about 1 in 600. In contrast, in families in which one child has IDDM, the likelihood of a second child coming down with IDDM is about 100 times higher. This latter high risk population seemed ideally suited to identify some of the factors that might precede the development of clinically apparent IDDM.

   In 1977, in collaboration with Dr. Fredda Ginsberg-Fellner at Mt. Sinai Hospital in New York, we initiated a long-term longitudinal study on families in which one child already had IDDM. When the proband was identified and the family admitted to the study, a variety of tests, including HLA typing, measurement of islet cell antibody, insulin secretion, C-peptide, and hemoglobin A_1c, were performed. Many of the tests were repeated at six-month to one-year intervals. Detailed data on the non-diabetic sibs who came down with diabetes were published in 1985. At that time, there were 178 families in the study and 351 non-diabetic sibs. During the period from 1977 to 1984, ten of the non-diabetic sibs developed IDDM. Most of the sibs who developed diabetes were HLA identical to their diabetic proband. Moreover, most of these children were islet cell antibody positive and showed impaired insulin secretion at the time they were admitted to the study, which was 2 to 74 months before clinically apparent diabetes.

   Since 1984, the number of families in the study increased from 178 to 328 and the number of originally non-diabetic sibs increased from 351 to 566. During that period, six more of the non-diabetic sibs came down with IDDM. This gives an overall prevalence of 11% for second cases of IDDM in our families. These additional children also showed a disproportionately high frequency of HLA identity to their diabetic probands, and most were islet cell antibody positive and showed impaired insulin secretion anywhere from 3 to 97 months before they became clinically diabetic.

We conclude, based on the insulin secretion data, that, at least in these families, impairment of beta cell function begins many months or years before the onset of clinically apparent diabetes, even in very young children. Moreover, the data show that the triad of HLA identity, islet cell antibody positivity and impaired insulin secretion are useful markers for identifying children at high risk of eventually coming down with IDDM. In fact, of six non-diabetic children who displayed this triad in 1984, two have now become diabetic. At the present time, of the 566 non-diabetic sibs, 18 are HLA identical to their diabetic probands, have islet cell antibodies and show impaired insulin secretion. It will be of interest to see what percentage of these children develop diabetes in the future. Although our ten-year longitudinal family study shows that islet cell antibodies and impaired insulin secretion precede the development of clinically apparent IDDM in most of the sibs, it does not tell us whether islet cell antibodies are the primary cause, a contributing factor, the result, or simply a marker for beta cell damage, nor does it tell us what triggers the autoimmune process.

IV. HERPES SIMPLEX VIRUS (HSV) AND OTHER PERSISTENT VIRAL INFECTIONS

1. Polyvalent Vaccine to Herpes Simplex Virus and Influenza: In collaboration with Dr. Bernard Moss at NIAID, we studied a double vaccinia virus recombinant which expresses both the HSV-1 glycoprotein D and the influenza A hemagglutinin. This recombinant is immunogenic in mice and protects against lethal challenge with either HSV or influenza. A potential limitation of polyvalent live vaccines, however, is existing immunity within the target population to certain of the expressed antigens. Such specific immunity could restrict replication of the vector, curtail expression of antigens, and reduce the total immune response to the vaccine. We have already demonstrated that preexisting immunity to the vector, vaccinia, results in decreased efficacy of vaccination with vaccinia recombinants. To determine whether preexisting immunity to HSV or influenza would affect vaccination with the vaccinia/HSV/influenza recombinant, mice were infected with a sublethal dose of either HSV or influenza, then vaccinated with respective single or double vaccinia recombinants, and then challenged with a lethal dose of HSV or influenza. We found that the vaccinia double recombinant (vaccinia/HSV/influenza) provided protection equivalent to either single recombinant, and was not adversely affected by preexisting immunity to HSV or influenza. These findings support continued investigation and development of live polyvalent vaccines.

2. Prevention of Reactivation of Herpes Simplex Virus: Previously, we described the development of a human model for studying

reactivation of HSV, using ultraviolet light and sunburn as a triggering stimulus. Using this model, we have initiated studies to examine the ability of various agents to block reactivation of HSV. The first study is a trial to determine if the antiviral agent acyclovir can prevent UV light-induced reactivation of HSV. In this study, we have entered over 30 patients and hope to complete it within the next three months. We have initiated a second study to examine the efficacy of sun blocking agents in preventing UV light-induced herpes labialis, and recruitment for that study has begun. In still another study, we have enrolled over 40 patients into a trial on the efficacy of oral acyclovir on the prevention of naturally occurring recurrent herpes labialis. Eight patients already have entered the treatment portion of that trial. We expect these latter two studies to continue over the next year.

V. TRANSGENIC MICE AND HUMAN IMMUNODEFICIENCY VIRUS (HIV)

To create a small animal model for HIV infection, in collaboration with Dr. Malcolm Martin of NIAID, we introduced an infectious HIV provirus into mouse embryos, using the microinjection technique. Of 13 founder animals obtained, six transmitted the transgene to their offspring. All 13 founder animals have remained healthy during 8 months of observation. However, serological analysis demonstrated antibodies against several HIV proteins in two of the founders (#13 and #61). Breeding of founder #13 resulted in litters in which all transgenic animals (14 of 37 offspring) developed obvious phenotypic abnormalities which included growth retardation, pulmonary perivascular lymphocytic infiltrates, a cutaneous syndrome resembling human psoriasis and lymphadenopathy. The affected animals developed symptoms between days 10 and 12 of life, and all died by day 30. Several tissues of line 13 animals were co-cultivated with a human cell line susceptible to HIV infection. Virus was isolated from spleen, lymph nodes and skin of affected animals. Immunoblot analysis of the recovered virus was indistinguishable from that of the parental HIV.

This is the first expression of an infectious viral genome in transgenic animals. Transgenic mice not only may be a useful system for studying the pathogenesis of AIDS and to assess potential therapies, but also may prove to be useful for studying the pathogenesis of other viral infections.

BIBLIOGRAPHY

Allaway GP, Srinivasappa J, Miller FW, Prabhakar, BS, Notkins AL. Autoantibody production by human B lymphocytes which spontaneously proliferate. In: Levine PH, Ablashi DV, Nonoyama M, Pearson GR, Glaser R, eds. Epstein-Barr virus and human diseases. Clifton, NJ: The Humana Press, 1987;287-91.

Allaway GP, Srinivasappa J, Miller FW, Prabhakar BS, Notkins AL. Spontaneously proliferating human B lymphocytes make autoantibodies. J Inf Dis 1988;157: 968-72.

Bahmanyar S, Srinivasappa J, Casali P, Fujinami RS, Oldstone MBA, Notkins AL. Antigenic mimicry between measles virus and human T lymphocytes. J Infect Dis 1987;156:526-7.

Burastero SE, Casali P. Characterization of human CD5 (Leu-1, OKT1)$^+$ B lymphocytes and the antibodies they produce. In: del Guercio P., ed. B cells and B cell products. Boca Raton: CRC Press, Inc. 1988;in press.

Casali P, Nakamura M, McChesney MB. Immunosuppression by measles virus. In: Friedman H, Specter S, Bendinelli M, eds. Handbook of viral suppression. New York: Plenum Press, 1988;345-73.

Casali P, Prabhakar BS, Notkins AL. Characterization of multireactive antibodies and identification of Leu-1$^+$ B lymphocytes as cells making antibodies binding multiple self and exogenous molecules. Int Rev Immunol 1988;3:17-45.

Casali P, Notkins AL. Probing the human B cell repertoire with EBV: Polyreactive antibodies and CD5$^+$ B lymphocytes. Ann Rev Immunol 1988,7:in press.

Ellis MM, Keller PM, Fyfe JA, Martin JL, Rooney JF, Straus SE, Lehrman SN, Barry DW. A clinical isolate of herpes simplex type II virus that induces a thymidine kinase with altered substrate specificity. Antimicrobial Agents and Chemotherapy 1987;31:1117-25.

Flexner C, Murphy B, Rooney J, Wohlenberg C, Yuferov V, Notkins A, Moss B. Successful vaccination with a polyvalent live vector despite existing immunity to an expressed antigen. Nature 1988;in press.

Garzelli C, Basolo F, Matteucci D, Prabhakar BS, Toniolo A. Picornavirus-induced immunosuppression. In: Friedman H, Specter S, Bendinelli M, eds. Handbook of viral suppression. New York: Marcel Dekker, Inc., 1988;in press.

Ginsberg-Fellner F, McEvoy RC, Rubinstein P, Notkins AL. Ten-year longitudinal study on children at high risk of developing IDDM. N Engl J Med 1987;317: 1352-3.

Hartman AB, Mallett CP, Srinivasappa J, Prabhakar BS, Notkins AL, Smith-Gill SJ. V_H family usage and binding analyses of polyreactive monoclonal autoantibodies derived from nonimmunized adult BALB/c mice. Current topics in microbiology and immunology 1988;137:211-5.

Hayashi T, Salata K, Kingman A, Notkins AL. Regulation of enzyme levels in the blood: Influence of environmental and genetic factors on enzyme clearance. Am J Pathol 1988;132:in press.

Inghirami G, Nakamura M, Balow JE, Notkins AL, Casali P. Model for studying virus attachment: Identification and quantitation of Epstein-Barr virus-binding cells by using biotinylated virus in flow cytometry. J Virol 1988;62:2453-63.

Klavinskis LS, Notkins AL, Oldstone MBA. Persistent viral infection of the thyroid gland. I. Alteration of thyroid function in the absence of tissue injury. Endocrinology 1988;122:567-75.

Klinman DM, Banks S, Hartman, A, Steinberg AD. Natural murine autoantibodies and conventional antibodies exhibit similar degrees of antigenic cross-reactivity. J Clin Invest 1988;82:652-7.

Lerman MI, Norman RL, Stevens L, Stinson SF, Saffiotti U. DNAase I-hypersensitive sites of the c-Ha-ras-1 protooncogene as targets for rapid binding and repair of benzo[a]pyrene adducts. In: Castellani A, ed. DNA damage and repair. New York: Plenum Press, 1987;in press.

Levine MM, Black RE, Ferreccio C, Clements ML, Lanata C, Rooney J, Germanier R, Chilean Typhoid Committee. Field trials of efficacy of attenuated Salmonella typhi oral vaccine strain Ty21a. In: Robbins J, ed. Bacterial vaccines. New York: Praeger Publishers, 1987;79-89.

Nakamura M, Burastero SE, Notkins AL, Casali P. Human monoclonal rheumatoid factor-like antibodies from CD5 (Leu-1)$^+$ B cells are polyreactive. J Immunol 1988;140:4180-6.

Oates EL, Allaway GP, Armstrong GR, Boyajian RA, Kerhl JH, Prabhakar BS. Human lymphocytes produce pro-opiomelanocortin gene-related transcripts. Effects of lymphotropic viruses. J Biol Chem 1988;263:10041-4.

Perna JJ, Mannix ML, Rooney JF, Notkins AL, Straus SE. Reactivation of latent herpes simplex virus infection by ultraviolet light: A human model. J Am Acad Dermatol 1987;17:473-8.

Prabhakar BS. Application of monoclonal antibodies to the study of Coxsackieviruses. In: Friedman H, Bendinelli M, eds. Coxsackieviruses - a general update. New York: Plenum Press, 1987;117-34.

Prabhakar BS, Srinivasappa J, Beisel KW, Notkins AL. Virus-induced autoimmunity: Cross reactivity of antiviral antibodies with self components. In: Schultheiss HP, ed., New concepts in viral heart disease. New York: Spring-Verlag, 1988;in press.

Puga A, Oates EL. Isolation and nucleotide sequence of rat Cu/Zn superoxide dismutase cDNA clones. Free Rad Res Comm 1987;3:337-46.

Rooney JF, Wohlenberg C, Cremer KJ, Moss B, Notkins AL. Immunization with a vaccinia virus recombinant expressing herpes simplex virus type 1 glycoprotein D: Long-term protection and effect of revaccination. J Virol 1988;62:1530-4.

Rooney JF, Wohlenberg C, Cremer KJ, Mackett M, Moss B, Notkins AL. A vaccinia virus recombinant which expresses the glycoprotein D gene of HSV-1: Duration of protection against HSV-1 challenge. Proceedings of the 15th International Congress of Chemotherapy 1988;in press.

Srinivasappa J, Garzelli C, Onodera T, Ray U, Notkins AL. Virus-induced thyroiditis. Endocrinology 1988;122:563-6.

Willey DE, Cantin EM, Hill LR, Moss B, Notkins AL, Openshaw H. Herpes simplex virus type 1 vaccinia virus recombinant expressing glycoprotein B: Protection from acute and latent infection. J Inf Dis 1988;in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00421-03

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Herpes simplex virus and persistent infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Rooney, James F.	Senior Staff Fellow	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR

COOPERATING UNITS *(if any)*

Laboratory of Viral Diseases, NIAID
Laboratory of Clinical Investigation, NIAID
Laboratory of Infectious Diseases, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.81	1.20	1.61

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ongoing studies in mice with a double recombinant vaccinia virus vector which expresses antigens from herpes simplex virus (HSV) and influenza A virus have demonstrated that vaccination with the double recombinant provides protection against challenge with either HSV or influenza A comparable to protection provided by respective single recombinants. In addition, vaccination with the double recombinant is not adversely affected by previous immunity to HSV or influenza virus. These findings support further research and development of polyvalent live recombinant vectors as vaccines for immunizing against multiple disease with single vaccination.

Clinical studies examining the ability of acyclovir to prevent ultraviolet light-induced reactivation of HSV have continued and a new study examining the ability of sun-blocking agents to prevent ultraviolet light-induced reactivation of HSV was started. A trial to determine the efficacy of daily oral acyclovir in preventing herpes labialis has progressed into the treatment phase of the study.

Prior studies with lactate dehydrogenase virus have indicated that impairment of enzyme clearance is an important factor in the regulation of serum enzyme levels. A critical step in the clearance of serum enzyme is the binding of enzymes to macrophages. Studies over the past year have demonstrated that this process can be studied using flow cytometry and that this technique can be used to study the binding of enzymes to cells, identify the cell type to which the enzyme binds and measure changes in the capacity of cells to bind enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00423 03

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Autoimmunity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prabhakar, Bellur S.	Microbiologist	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR
Srinivasappa, Javaraiah	Visiting Associate	LOM NIDR
Vivino, Alfred A.	Staff Fellow	LOM NIDR
Mishoe, Helena	Expert	LOM NIDR
Allaway, G.P.	Visiting Associate	LOM NIDR
Coutelier, Jean P.	Visiting Associate	LOM NIDR
Shibata, Motohiro	Visiting Fellow	LOM NIDR

COOPERATING UNITS *(if any)*
NIDDK, NIH, Bethesda, Maryland 20892

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
15.45	11.09	4.36

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNA clones were isolated by immunoscreening a human thyroid carcinoma gt11 expression library with immunoglobulins purified from serum of a patient with autoimmune Graves' disease. One clone (ML8) containing a 1.25 kb insert identified a 2 kb mRNA in human thyroid and thymus tissues and human lymphocytes but not in human brain, pituitary, liver, placenta and thymus epithelium. Using the 650 bp 5' end of the ML8 clone as a probe, a full length 2.05+0.05 kb human cDNA was obtained and sequenced. This cDNA encodes a 69,812 dalton (70kd) protein with two potential N-linked glycosylation sites and a hydrophobic region compatible with a membrane spanning domain. The size of the protein synthesized in vitro from this cDNA using sp6 polymerase and in the baculovirus expression system corresponds to the size deduced from the nucleotide sequence. The protein reacted with sera from several patients with Graves' disease but, not in general, with sera from normal controls. This suggested that the 70 kd protein is a thyroid autoantigen. The functional significance of the transcript in thyroid cells and immune system is currently unknown, but it is of interest that lymphocytes and thyroid are reputed to have TSH binding sites. Studies are underway to determine whether the 70 kd protein is the receptor for TSH and/or an autoantigen involved in the pathogenesis of Graves' disease.

Similarly, a cDNA expression library prepared with mRNA from enriched human islets was screened with diabetic patient sera. One of the human clones designated HIDEN 10 contains a 1 kb cDNA that hybridizes with a 1 kb transcript and encodes a novel protein of 212 amino acids. In addition, several cDNA clones from a rat insulinoma library have been obtained, using a monoclonal anti-islet cell antibody. Partial sequence of these clones indicate that they are novel and are being further analyzed. Our studies should make it possible to use some of these autoantigens in assays for early detection of autoantibodies.

PHS 6040 (Rev. 1/84)

PROJECT NUMBER

Z01 DE 00423 03

Continuation of Principal Investigator List

Takai, Osamu	Visiting Fellow	LOM NIDR
Nakayama, Hiroyuki	Visiting Fellow	LOM NIDR
Gong, Yu	Visiting Associate	LOM NIDR
Oates, Edward L.	Staff Fellow	LOM NIDR
Chan, John	Visiting Associate	LOM NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00467-01

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human B cell repertoire and autoantibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Casali, Paolo	Visiting Scientist	LOM NIDR
Ueki, Yuji	Visiting Fellow	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR
Burastero, Samuele E.	Visiting Fellow	LOM NIDR
Harindranath, Nagaradona	Visiting Fellow	LOM NIDR
Goldfarb, Inna	Visiting Associate	LOM NIDR
Jackson, William L.	Medical Staff Fellow	LOM NIDR
Dhawan, Subhash	Senior Staff Fellow	LOM NIDR

COOPERATING UNITS *(if any)*

Mount Sinai School of Medicine, New York, New York
Texas Southwestern Medical School, Dallas, Texas
Cetus Company, Palo Alto, California

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.66	6.75	2.91

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using limiting dilution culture, EBV transformation and somatic hybridization techniques, cell lines capable of making human monoclonal antibodies to a number of self-antigens, e.g., ssDNA, thyroglobulin, insulin, Fc fragment of IgG, and exogenous antigens, e.g., tetanus toxoid, HIV, have been established.

These technologies have been applied to the study of the human B cell repertoire in patients with systemic lupus erythematosus, rheumatoid arthritis and Hashimoto's disease. B lymphocytes producing two groups of autoantibodies were detected in these patients. The first one includes autoantibodies binding to multiple self and exogenous antigens, in general, with low affinity - polyreactive antibodies which can be detected also in healthy subjects. The second one includes high affinity monoreactive autoantibodies and is exclusive of autoimmune patients. Using fluoresence-activated cell sorter, the B lymphocytes devoted to the production of polyreactive "autoantibodies" were identified as a discrete ($CD5^+$) B cell subset.

The role of $CD5^+$ B lymphocytes and that of other B cells in the human immune response to rabies virus have been investigated. It was found that the B cells recruited during a primary antibody response are committed to the production of polyreactive antibodies, mainly of the IgM class, and are indeed $CD5^+$. In sharp contrast, the B cells recruited in a secondary or tertiary immunization belong to the CD5- compartment and produce antibodies, mainly IgG, which are monoreactive and specific for the inducing antigen. The difference in affinity between the "primary" antibodies and "secondary" antibodies can be as much as 1,000 times. Current experiments will possibly clarify whether this tremendous enhancement in affinity is achieved through accumulation of somatic point mutations and/or rearrangement of different gene segments coding for the regions of the antibodies.

ROJECT # Z01 DE 00467-01 LOM -- PRINCIPAL INVESTIGATOR (Cont'd)

ishishita, M. Visiting Associate LOM NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00471 01

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transgenic mice as a model for studies on the pathogenesis of AIDS and autoimmunity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Abramczuk, Jan W.	Visiting Scientist	LOM NIDR
Dickie, Peter	Visiting Associate	LOM NIDR
Dillon, Patrick J.	IRTA Fellow	LOM NIDR
Rooney, James F.	Senior Staff Fellow	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR

COOPERATING UNITS *(if any)*

Laboratory of Molecular Microbiology, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.61	3.80	1.81

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A model of the acquired immunodeficiency syndrome (AIDS) has been created through the introduction of the human immunodeficiency virus 1 (HIV-1) proviral DNA into the mouse germ line. One of the HIV transgenic founder animals have produced offspring which manifest several symptoms consistent with human AIDS. These are: retardation of growth, skin lesions resembling pronounced epidermal proliferation, pulmonary perivascular lymphocytic infiltrates, and lymphadenopathy. All the affected animals die by day 30 of life. The infectious HIV has been recovered from some tissues of the diseased animals. These results show that the HIV transgenic mouse system can be used as a valuable tool in eliciting the pathogenesis of AIDS, and testing potential treatments for this disease.

To study the effects of autoimmune response to a pre-determined immunogen, we have created transgenic mice carrying the structural gene of the herpes virus 1 glycoprotein D (gD). The introduced gD construct contains, as a regulatory element, the insulin promoter (pIPEgD), or SV40 promoter (pSVgD). We have expected that the expression of gD in the pancreatic beta cells (pIPEgD transgenics), or in a broad range of other tissues (pSVgD transgenics) will induce the immune responses, at least in some cases. At this stage, animals from only one pSVgD transgenic line have been shown to mount spontaneous anti-gD antibodies. No apparent abnormalities have been observed in these animals. Two other pSVgD lines appear to be tolerant to gD. We intend to identify the tissue localization of gD expression, and look for the effects of augmenting the immune response with recombinant vaccinia-gD, or of breaking the state of tolerance.

EPIDEMIOLOGY AND ORAL DISEASES PREVENTION PROGRAM

ANNUAL REPORT OF THE ACTING DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH

On numerous occasions this past year, the commemoration of the 40th Anniversary of the National Institute of Dental Research (NIDR) has called attention to the past and ongoing contributions of Epidemiology and Oral Disease Prevention Program (EODPP) intramural scientists and support staff to a better understanding of the magnitude, sources, natural history, and impacts of oral diseases and disorders. During this same period of time, attention has also focussed on the centrality of epidemiology and biological analysis, and on the need for collaboration between the public and private sectors, in efforts to improve the oral health of the United States population.

Looking back over the past year, these Program emphases have been most visible in the large-scale epidemiologic studies which have been reported on for adults and completed for U.S. schoolchildren; in the efforts which have gone into the development of dental components for what are sure to become landmark studies, including the 1988-94 National Health and Nutrition Examination Survey (NHANES III), and the Walter Reed Army Medical Center's natural history study of HIV infection; and in the development of a "Research and Action Program for Improving the Oral Health of Adults and Older Americans."

This latter NIDR-wide initiative, for which EODPP scientists have so far played the key staff function, is designed to spur the basic and clinical research necessary to drive nation-wide public and private sector actions to improve the oral health of all Americans. The explicit goal of the program is to eliminate tooth loss in the U.S. population and prevent further deterioration of the oral health status of individuals who have a compromised dentition. The NIDR role will be to expand the knowledge base and accelerate the transfer of laboratory findings to the public and to the profession. The Institute is emphasizing research on normal changes in the oral tissues and the body's defense mechanisms in aging, and how these changes are affected by disease, diet, medications, environment, and lifestyle. Epidemiologic and behavioral science research will be essential to document the prevalence of oral diseases in special populations (e.g., the frail, the handicapped), identify risk factors, advance prevention research, and monitor science transfer. Historically, this research and action program may be viewed as an attempt to do for current and future generations what Dr. H. Trendley Dean and his colleagues did for all of us.

If this current 40th anniversary year has provided an opportunity to look back at the seminal research and action orientation of Dr. H. Trendley Dean and his colleagues, it has provided an even better context for looking forward, and for coaligning the Program in accord with a vision of the future. Accordingly, during this past year the EODPP has undergone a structural reorganization designed to increase the responsiveness of the Program to the needs for oral epidemiology and science transfer in the 1990's and beyond--selected aspects of which are described below and in the accompanying Branch reports.

Organization of Report

The remainder of this report is organized into four main sections. The first section describes the key aspects of EODPP's new mission statement. Program highlights for each of the three major Branches are outlined in the second section. The third and fourth sections provide detailed reports for those sections of the Program which are now located within the Office of the Director; namely, the Office of Program Support and the Microbial Systematics Section.

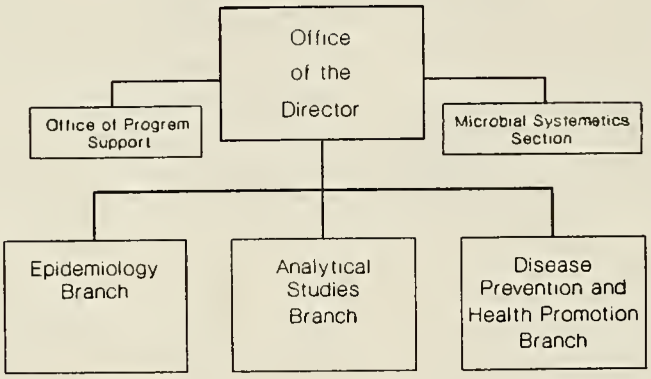
EODPP MISSION

The overall mission of the Epidemiology and Oral Disease Prevention Program (EODPP) includes the following key interrelated objectives:

- To plan, develop, direct, and apply epidemiologic methodologies to investigation of oral diseases and disorders.
- To plan and conduct research analyzing the effect of changing oral disease patterns on dental education, research and delivery systems.
- To plan and conduct clinical, field, demonstration, and related studies in the prevention of oral diseases.
- To plan and coordinate health education and promotional activities related to the prevention of oral diseases and disorders, targeted to the general public, health professionals and the scientific community.
- To design, develop and coordinate programs which facilitate or implement the transfer of research findings to application.

During this past year, EODPP's activities in pursuit of these objectives were reorganized according to the schema shown below. In some instances, further details on the internal structure of these Program components is provided in the narrative which follows or in the separate Branch reports.

EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM


Office of the Director
Office of Program Support
Microbial Systematics Section
Epidemiology Branch
Analytical Studies Branch
Disease Prevention and Health Promotion Branch

HIGHLIGHTS OF BRANCH ACTIVITIES

The first major section of this report highlights significant activities and achievements of the three Branches over the past year.

Epidemiology Branch

The Epidemiology Branch plans, conducts, and directs a coordinated program of epidemiologic research on oral diseases and conditions, craniofacial diseases and disorders, and pain syndromes. This program includes studies designed to monitor the incidence and prevalence of oral deseases and conditions, as well as studies designed to explore the role and relative importance of major risk factors, and the natural history of oral diseases and conditions.

The Branch is organized formally into three sections: (1) a Caries Section, (2) a Periodontal Diseases Section, and (3) a Soft Tissue, Craniofacial Defects and Pain Section.

Notable activities which staff in each of these sections of the Epidemiology Branch have carried out this past year include the following:

- Analysis of data and reporting of results from the 1986-87 National Survey of the Oral Health of U.S. Schoolchildren which showed that the decline in dental caries, first reported in 1981, has continued at approximately a constant rate.

- Analysis of region-specific results from the 1985-86 Survey of the Oral Health of U.S. Employed Adults and Seniors, and the preparation of a monograph presenting the results of these analyses.

- Completion of final examinations in a longitudinal study of the incidence of incipient periodontal disease in Navajo Indian adolescents.

- Continuation of longitudinal studies of the natural history of periodontal disease in Sri Lankan tea laborers, and of the stability of gingival lesions over time and their conversion to a progressive loss of attachment in Norway.

- Initiation of research in relatively new areas of oral epidemiology, including studies of oral mucosal lesions, traumatic injuries to teeth, and the oral manifestations of HIV infection.

- As part of the Walter Reed Army Medical Center's longitudinal study of the natural history of HIV infection, a dental component has been developed and incorporated into the research protocol.

- A major dental component has been developed for the 1988-94 National Health and Nutrition Examination Survey (NHANES III) which went into the field in the first quarter of FY 89. An automated data entry system has been developed for the dental component of NHANES III and will be introduced into the field data collection procedures as soon as pretesting has been completed.

- Participation in two pain workshops; one sponsored by the National Center for Health Statistics (NCHS) to evaluate the implications of recent advances in the epidemiology of pain for NCHS data systems; the other sponsored by the University of Washington Multidisciplinary Pain Center to evaluate the current state of the science of pain measurement.

Analytical Studies Branch

The Analytical Studies Branch conducts research aimed at clarifying the manifold interrelationships among patterns of oral diseases and disorders; the need for, and utilization of, dental care; dental education, dental research, and dental delivery systems.

Central to the work of the Analytical Studies Branch this past year have been the following activities:

- Analyses of the prevalence, severity, and extent of periodontal diseases in the United States, including the role of periodontal diseases in tooth mortality.
- The developement of estimates of periodontal treatment needs in the United States based on epidemiological data.
- A comparative study of the costs for dental services and dentists' incomes in the United States and Norway.
- Analysis of the relationships among aging, nutritional status and bone loss.
- Development of an NIDR research protocol to study the utilization of dental services, treatment needs, costs, and dental diseases among a panel of healthy veterans (being studied longitudinally by the Boston Veterans Administration).
- Development of the diagnostic criteria for the evaluation of restorations, tooth conditions, and prostheses in the oral examination component of NHANES III.

Disease Prevention and Health Promotion Branch

The Disease Prevention and Health Promotion Branch plans and directs clinical research to identify, develop, and evaluate methods for the control and prevention of oral diseases and conditions. Fundamental to the work of the Branch is a broad spectrum of research and action initiatives designed to improve the oral health of all Americans through educational programs and other forms of science transfer.

During this past year, staff activities and achievements have included the following:

- Coordination of NIDR and outside activities related to the development of a research and action program for improving the oral health of U.S. adults and older persons.

- Analyses of trends in fluorosis among Illinois schoolchildren living in areas with water fluoride concentrations of 1, 2, 3, and 4 times the optimal level.
- Evaluation of the effects of certain combinations of fluoride procedures in conjunction with dental sealant therapy in preventing dental caries.
- Evaluation of the comparative effectiveness of plaque control and the elimination of gingival bleeding as motivational tools for long-term maintenance of optimal oral hygiene.
- An evaluation of the use of stannous fluoride to prevent the development of plaque and reduce gingivitis among adults.
- Provision of technical assistance to state and local communities on the use of community water fluoridation as a method for preventing dental caries.
- Development of an inventory of existing national oral health and dental databases.
- Compilation of an inventory of existing oral health education and promotion programs and activities.
- Development and evaluation of self-report items on oral health, dental care, and orofacial pain for inclusion in NHANES III and in the 1989 National Health Interview Survey.
- Consultation and assistance to educational and health agencies in the U.S. and abroad regarding oral disease prevention and health promotion.

In the later part of the year, the activities of the Branch were notably enhanced with the addition of contributed staff from other agencies. In August, Dr. Steven Corbin, a Disease Prevention Policy Analyst with the Centers for Disease Control, was assigned to the Disease Prevention and Health Promotion Branch to work on Public Health Service wide aspects of the development and coordination of the "Research and Action Program for Improving the Oral Health of Adults and Older Americans." In September, Dr. Robert Selwitz, a Disease Prevention Research Specialist with the Health Resources and Services Administration, was assigned to the Disease Prevention Section of the Branch to work on the development and coordination of research projects related to this research and action program.

OFFICE OF PROGRAM SUPPORT

The Office of Program Support conducts and directs a broad spectrum of scientific, technical, and administrative activities in support of program needs. These activities are carried out within three functional workgroups: (1) a biometry unit, (2) a data services unit, and (3) a contract and reports unit.

Biometry Unit

The primary activities of this unit over the past year have been in the conduct of methodologically-oriented research. Most of this research has focussed upon the application of known, or the derivation and/or modification of new, statistical methods for analyzing dental epidemiologic data. Specific projects during this past year have included the following:

- The review and development of sensitive statistical methods for analyzing longitudinal periodontal measurements.

- The development and evaluation of specific types of caries prediction methodologies, including the estimation of average DMFS or DMFT scores for a community based only on the knowledge of age-specific caries-free percentages, the development of prediction models useful for explaining the relationship among the most important caries prediction variables, as well as logistic regression models useful for predicting which individuals may be at "higher" risk of developing dental caries.

- Growth curve models useful for describing growth rates of specific clinical phenomena (such as microorganism levels) over time.

Biometry Unit staff are also involved in a broad spectrum of consulting activities. Current efforts in this area of their work include consulting activities within the Institute related to the following studies:

- An epidemiologic measles study in the Philippines

- An epidemiologic periodontal study among American Indians

- A clinical trial comparing bleeding versus plaque control strategies for preventing gingivitis

- A demonstration study of the effectiveness of sealants in combination with various fluoride modalities

Consulting activities outside the NIDR have also continued, and include work with the American Dental Assocation Council on Dental Therapeutics; lecturing at the Bethesda Naval Dental School; and collaborations with faculty at Columbia University and the University of Goteborg, Sweden.

Publications:

Heifetz, S.B., Driscoll, W.S., Horowitz, H.S., and Kingman, A. Prevalence of dental caries and dental fluorosis in areas with optimal and above-optimal water fluoride concentrations: a 5-year follow-up survey. JADA, 116:490-495, 1988.

Kingman, A., Little, W.A., Gomez, E., Driscoll, W.S., Heifetz, S.B., Sheats, R., and Supan, P. Associations of streptococcus mutans and lactobacillus and dental caries in a U. S. adolescent population. Community Dent Oral Epidemiol, 16:98-103, 1988.

Schreiber, C.T., Shern, R.J., Chow, L.C. and Kingman, A. Effects of rinses with an acidic calcium phosphate solution on fluoride uptake, caries, and in situ plaque pH in rats. JDR, 67:959-963, 1988.

Presentations:

Kingman, A., Morrison, E., Löe, H., and Smith, J. "Systematic Errors in Estimating Prevalence and Severity of Periodontal Disease," IADR Meeting, Montreal, Canada, 1988.

Kingman, A. "Methods for Caries Prediction and Identifying Subjects at High Risk," Annual ORCA Meeting, Angers, France, 1988.

Data Services Unit

The bulk of data processing activities this past year was carried out in connection with the final editing and data reduction of information obtained through the National Survey of Oral Health of U.S. Schoolchildren, 1986-87. Final cleanup of the master file of dental caries measurements on 40,000 children was accomplished, and collaboration with contractor staff resolved age and sex identification problems which arose in matching individual data records from different parts of the survey. Weights for use in producing population estimates from the sample data were constructed by the contractor, added to the file, and initial analysis of dental caries data was performed by May, 1988.

Since then the staff of the Data Services unit have been working on the preparation of tabular and graphic materials for a monograph on dental caries in schoolchildren.

As part of the 1986-87 survey of schoolchildren a new system was implemented for collecting, processing and editing periodontal measurements collected in this survey. Namely, whole mouth indices for gingivitis, calculus and loss of attachment were done. Microbiological readings from saliva samples, collected on a subsample of about 2,000 children in the survey, were also keyed and checked for accuracy. Information on presence of soft tissue lesions in about 1,800 schoolchildren was scanned, edited and readied for analysis.

In addition to the materials from the children's survey material, the editing and proofing of materials for a regional monograph based on the adult survey was accomplished this past Spring. Tables, charts and graphs from both studies were also designed and prepared as slides or overhead transparencies for use in presentations by numerous investigators.

Data Services Unit staff also worked on projects for the Minnesota and West Virginia State Health Departments, as well as for a county health department in Texas. Dental examination data from epidemiologic studies of mothers and children, collected under contract in Alabama and Maine, were also processed and made ready for analysis. School rosters being used to track sample members of longitudinal clinical studies in Virginia and Ohio were also updated numerous times in response to investigator needs.

Throughout the year, staff participated in training and calibration activities in preparation for the 1988-1994 National Health and Nutrition Examination Survey (NHANES III) being conducted by the NCHS.

Contracts and Reports Unit

The primary activities of this unit over the past year have been in the area of contract development and administration. Research has continued on the development of intraoral controlled-release delivery systems for the prevention and treatment of oral disease. The manufacturing method for the Intraoral Fluoride Releasing Device (IFRD) has been improved through a contract with the Southern Research Institute so that it is now possible to produce large quantities of IFRDs for clinical studies at a reasonable cost. New holding systems for retaining and protecting IFRD in the mouth have been developed under a contract with the Eastman Dental Center. These new systems are scheduled to undergo six months of clinical evaluation in adolescents beginning in September 1988. If found to be safe and functional, the new holders could be used in a long-term trial of the efficacy of IFRD for the prevention of dental caries. The holders also should be suitable for retaining controlled-release pellets for additional therapeutic agents for the treatment of oral diseases in addition to caries. Laboratory studies in the EODPP have shown that chlorhexidine and tetracycline can be released in a controlled manner from pellets fabricated similar to the IFRD.

A request for proposals for the development of a bioadhesive sustained-release delivery system for the treatment of oral candidiasis has been prepared and was issued in September 1988. Such a system could be useful in treating oral fungal infections in immunocompromised individuals.

A collaborative project has been initiated with the Diagnostic Systems Branch, NIDR, to investigate the effect of flurbiprofen, a non-steroidal anti-inflammatory agent, on the rate of alveolar bone loss in progressive periodontitis. An Investigational New Drug Application (IND) for this double-blind placebo-controlled trial was prepared and filed with the FDA. Clinical treatment is scheduled to begin in October 1988 and last for up to 12 months.

The initial pilot studies for the clinical studies of periodontal disease in older adults have been completed by contractors at the University of Texas Health Science Center at San Antonio and the State University of New York at Buffalo. A second pilot study is presently being conducted to refine procedures with full scale clinical studies scheduled to begin in the Spring of 1989.

Publications:

Mirth, D.B. Controlled-release therapeutic systems: technology applicable to the treatment of oral disease. Adv Dent Res 1:109-118, 1987.

Shern, R.J., Little, W.A., Kennedy, J.B., and Mirth, D.B. Effects of octenidine on dental plaque and gingivitis in monkeys. J Periodontol 58:628-633, 1987.

Presentations:

Mirth, D.B. "Development of Controlled Resease Delivery Systems for Treatment of Oral Disease," NIDR Fellowship Rounds, Bethesda, MD, March 3, 1988.

Mirth, D.B. "Applications of Controlled Release in Dental Therapeutics," Pharmaceutical Chemistry Department Seminar, The University of Kansas, Lawrence, Kansas, April 21, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00282-09 EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Refinement of the Intraoral Fluoride Releasing Device

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mirth, Dale B.	Research Chemist	EODPP, OPS, NIDR

COOPERATING UNITS (if any)

Eastman Dental Center, Rochester, New York 14620
Southern Research Institute, Birmingham, Alabama 35255

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of Program Support

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
	0.2	

CHECK APPROPRIATE BOXES

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Intraoral Fluoride Releasing Device (IFRD) is a new intraoral therapeutic system being developed by the National Institute of Dental Research. The IFRD is designed to provide continual topical fluoride for periods of up to six months for the prevention of dental caries. The IFRD reduced the incidence of experimental dental caries in rats by more than 50% and has been used in humans for periods of up to six months without producing adverse effects.

The present objectives of this project are: (1) to develop an improved manufacturing method for the production of IFRDs for clinical use, and (2) to refine the shape and method of attachment of the IFRD in order to make it more durable and easier to use in humans.

Progress has been made in improving the manufacturing process and it should now be possible to produce IFRDs for use in human trials at a reasonable cost. Several new methods of retaining the IFRD in the mouth have been developed and will be clinically evaluated in adolescents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00417-03 EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intraoral therapeutic systems for periodontitis and AIDS-related infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Mirth, Dale B.	Research Chemist	EODPP, OPS, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of Program Support

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to develop intraoral controlled-release therapeutic systems for antibiotics/antimicrobial and antifungal agents for the treatment of periodontal disease, AIDS-related oral diseases and other opportunistic oral mycotic infections.

Biocompatible copolymers of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) have been used to produce a membrane-controlled delivery system for tetracycline that should be suitable for short-term intraoral treatment of periodontal disease. Results with this system have shown that the hydrogel copolymers used in the Intraoral Fluoride Releasing Device also can be used for the delivery of large organic molecules *in vivo*. In a study in primates, ten days of treatment with tetracycline controlled-release pellets releasing 0.4 to 1.0 mg of tetracycline per day produced significant decreases in crevicular fluid flow, number of bleeding sites on probing, and bacterial morphotypes associated with periodontal disease in subgingival plaque samples.

Prototype controlled-release pellets for chlorhexidine have been fabricated using the same biocompatible copolymers used with the tetracycline and fluoride systems. These pellets released biologically active chlorhexidine for 30 days.

It should be possible to use this technology to develop intraoral controlled-release delivery systems for the lower molecular weight antifungal agents. Such systems could be beneficial in immunocompromised individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00418-03

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methods for Analyzing Longitudinal Periodontal Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of Program Support

SECTION

Biometrics Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An investigation was made regarding the degree of bias and relative bias in estimating the prevalence and severity of periodontal disease that results from employing partial mouth examinations. The partial mouth exams that were considered were all based on the random half-mouth technique. This technique consists of examining sites in two quadrants, one randomly selected from the maxillary arch and one from the mandibular arch.

Three large epidemiologic data sets in which full mouth periodontal measurements were collected were used in this study.

True prevalence and severity of the disease was based on the full-mouth examination. This consisted of diagnosing 112 sites, based on four sites for each of the 28 teeth (excluding third molars). The sites examined were the mesiobuccal, midbuccal, distobuccal and midlingual.

Four separate partial-mouth examinations were considered: the 1st evaluated mesial sites only (M), the 2nd the mesiobuccal and midbuccal sites (MB), the 3rd the mesiobuccal, midbuccal and distobuccal sites (MBD), and the 4th the mesiobuccal, midbuccal, distobuccal, and midlingual sites (MBDL).

All four partial-mouth examinations produced underestimates of the prevalence of periodontal disease, whereas the estimates of severity were dependent upon which sites were selected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00435-02

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Systematic Errors in Assessing Periodontal Disease Using Partial Scoring Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Statistician (Health)	OPS, EODPP, NIDR
Morrison, Edith C.	Senior Staff Fellow	OPS, EODPP, NIDR
Löe, Harald	Director	NIDR
Smith, Jacqueline	Programmer	OPS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Program Support

SECTION
Biometrics Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.30	.30	.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Statistical methods are presented for analyzing longitudinal periodontal loss of attachment data using subject based summary measures. The methods are illustrated by sing data from a 2-year clinical study in which a conservative periodontal therapy was evaluated. The 2-year study period was divided into the 1st 6-month period (treatment period) and the 2nd 18 months (maintenance period). Individual sites within patients were classified by their initial probing pocket depth values: shallow, moderate or deep. Treatment and maintenance effects were assessed by using multivariate statistical methods (Hotelling T-square tests) jointly, and for each class of sites, separately.

For this data set it was shown that this conservative therapy produced significant improvement for deep sites, minor improvement for moderate sites, and significant deterioration for shallow sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00436-02

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Repeated Measurement Analysis of Microbiological Changes in Dental Plaque

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Li, Shou-Hua	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Program Support

SECTION
Biometrics Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is common in the laboratory to measure microbiological changes in dental plaque in subjects (animals) over periods of time. It is usually assumed that the repeated measurements from each subject are correlated rather than independent observations. The statistical procedures when correlation is present in the repeated measurements are different from those used under the independence model. There are three different statistical approaches that may be used in this situation. The simplest method is to use the univariate analysis of variance approach. The second approach is based on the multivariate analysis of variance technique. A third approach is the growth curve analysis. The growth curve analysis is based on a separate polynomial growth curve of time that is fit to each group of data. Data on microbiological changes in dental plaque from three groups of rats measured repeatedly over time were utilized to illustrate three types of analyses of repeated measurements. This study focuses on how the growth curve method can be successfully applied to analysis of microbial growth data in dental plaque since this approach has not been used by dental researchers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00461-01

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methods for Caries Prediction and Identifying Subjects at High Risk

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Program Support

SECTION
Biometrics Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from a recently conducted longitudinal caries study were used to assess the predictive value of specific groups of variables in predicting the level of dental caries incidence in adolescent subjects. The data available included demographic information, use of topical and systemic fluoride, initial caries scores, microbiologic levels of S. mutans and Lactobacilli on three occasions, and multiple 24-hour recall dietary intake information.

These data were analyzed from two perspectives: 1) to assess the predictive value of predicting caries incidence over the whole range of caries experiences on the log scale; and 2) evaluate the predictive value of selected variables to identify subjects at high risk.

The initial caries scores together with the microbiologic counts were the best predictors of caries incidence for the group. The initial caries scores, the microbiologic counts and the use of fluoride prophylaxes were the best fitting variables in the logistic models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00462-01

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methods for Estimating Age-Specific DMFT and DMFS Scores in the U. S.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Li, Shou-Hua	Statistician (Health)	OPS, EODPP, NIDR
Kingman, Albert	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of Program Support

SECTION
Biometrics Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.3	.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An investigation was conducted in which the age-specific prevalence of DMFT and DMFS scores could be made for U. S. subjects (adults and children) by simply using the corresponding percentage of caries free subjects.

Knutson's model, a modification of Knutson's model and various logistic models were compared and evaluated for the estimation of age-specific DMFT and DMFS scores. The National Caries Prevalence Surveys (1980 Children's Survey and the 1985 Adult Survey) provided the caries data to be modeled. Age groupings were formed for the adult data set up to the age of 35 years. Data for adults older than 35 were not used, since the DMFT is not caries specific for adults.

MICROBIAL SYSTEMATICS SECTION

The Microbial Systematics Section (MSS) has three main areas of activity: (1) international communication of microbial and cell line clone data; (2) development of methods of computer management and analysis of such data; and (3) studies of microbial systematics and ecology. The MS Section has been active in all three areas this year.

International Communication

Various members of the MSS have participated in the establishment of the Microbial Strain Data Network (MSDN) which is a collaborative initiative of three components of the International Council of Scientific Unions (ICSU): Committee on Data for Science and Technology (CODATA), World Federation for Culture Collections (WFCC), and International Union of Microbiological Societies (IUMS). The Chief, MSS, chairs the MSDN Committee of Management which is the policy and design oversight body. MSS staff are active on the Technical (i.e, Network operations and software development) and Central Directory (i.e., Network main database) Committees. The Information Officer, MSDN, works with the MSS Staff to design and implement the electronic mail, database management, and computer conferencing facilities of the MSDN. These facilities are now operational worldwide on the international telecommunications networks (PSS). They have been used by the Epidemiology Branch in aiding communication during the design of a multisite study of periodontal disease in older adults. MSS staff collaborated in design and as faculty members for MSDN training courses on use of microcomputers in interlaboratory communication, culture collection management, and strain data analysis. These courses are sponsored by the United Nations Environment Programme. Courses have been held in Czechoslovakia and Brazil. Venues for courses in the upcoming two years are USA, Egypt, and USSR, Guatemala, PRC, and India.

The MSS actively participates in the Hybridoma Data Bank, an initiative of CODATA and the International Union of Immunological Societies. The Chief, MSS, is a member of the HDB Task Group. Staff of the MSS are responsible for much of the software and database design. The HDB also uses the electronic communication network described above to communicate among its three nodes located at the American Type Culture Collection, Rockville, MD, at the Institute for Chemical and Physical Research (RIKEN), Saitama, Japan, and at the Medical School, University of Nice, Nice, France.

Methods Development

The MSS, in collaboration with many microbiologists around the world, has an ongoing project of developing a unified coding system for computer management of microbial information. The current version of this system is published in book form and is installed as a reference database on the aforementioned MSDN system. As such, the database forms the controlled vocabulary for the MSDN Central Directory of Collections of Strain Data. Further, the coding system is being accepted as an international standard for communicating strain data among microbiologists.

Design and programming of a comprehensive suite of computer programs, the Microbial Information System (MICRO-IS), is a long term, ongoing project of the MSS. A main-frame version of the MICRO-IS is currently used extensively by the MSS for management of strain data. Additionally, the FDA and EPA use the system for managing and analyzing microbial data in their regulatory roles. The latest thrust of these methodological efforts is development of a portable version of the MICRO-IS for installation on a wide-range of computers including personal computers, mini-computers, and main-frames.

A program for conversion of controlled vocabulary information in text records of the HDB into the highly compressed, table oriented MICRO-IS format has been implemented by the MSS for the HDB. The conversion process also has extensive spelling, syntax, and format error checking capabilities. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required if we are to have any realistic chance of computerizing valuable archival records of phenotypic strain data.

Microbial Systematics and Ecology

The MSS continued the collaboration with the International Working Group on Mycobacterial Taxonomy to elucidate the taxonomic relationships within this genus of pathogens and saprophytes by providing computer analysis of the phenotypic data submitted by the cooperating reference laboratories. The latest findings are yielding new criteria for the separation of the AIDS patient infective M. avium from the closely related non-infective M. intracellulare.

The MSS is continuing collaboration with the Center for Veterinary Medicine, FDA, on data concerning the results of use of antibiotics as growth promotants for commercial meat animals.

With staff of the EPA and ATCC, the MSS is establishing resources for use in risk assessment of release of genetically engineered organisms in the environment. These resources include accession and analysis of databases of phenotypic characteristics of microorganisms known to be used in genetic manipulation and biotechnological processes, analysis of problems in accession and standardization of such databases from diverse sources, and redefinition of taxonomic boundaries of such organisms. The redefinition is critical to the design of the computer registry for such organisms under the Toxic Substances Control Act.

Staff of the MSS are continuing analysis of phenotypic data on oral microbiota to improve taxonomy and identification criteria for these organisms.

Publications:

Krichevsky, M.I. "Clones: Coding, Computing and Communicating" in Biotechnology Information '86. R. Wakeford (ed.). IRL Press, Oxford, 1987.

Krichevsky, M.I., Sugawara, H., and Fabricius, B.-O. "Culture Collections as Information Resources for Biotechnology" in Living Resources for Biotechnology. B. Kirsop and D. Hawksworth (eds.). Cambridge University Press, in press.

Walczak, C.A. and Krichevsky, M.I. "An Opinionated Overview of Information Needs in Biotechnology" in Piecing the Puzzle Together: A Conference on Integrating Data for Decision Making, in press.

Krichevsky, M.I. and Walczak, C.A. "Establishing a Meaningful Relationship with Your Computer" in Microbial Technology in the Developing World. E.J. DaSilva, Y.R.. Dommergues, E.J. Nyns, and C. Ratledge (eds.). Oxford University Press, 1987.

Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P., and Truper, H.G. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37:463-464, 1987.

Jong, S.-C., Holloway, L., McManus, C., Krichevksy, M.I., and Rogosa, M. Coding of strain features for computer-aided identification of yeasts. *Mycotaxon* 31:207-219, 1988.

Krichevsky, M.I. "Global Interfacing of People, Places, Data, and Knowledge: Calm Sea and Prosperous Voyage?" in Biomolecular Data: a Resource in Transition. R.R. Colwell and D.G. Swartz (eds.). Oxford University Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00044-18

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Handling of Microbial Strain Information by Computers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, EODPP, NIDR

COOPERATING UNITS *(if any)*
See Attachment

LAB/BRANCH

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Coding conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

The Microbial Information System programs being developed in a related project are being used to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, and ecological uses. The long term goal is to establish a worldwide data network at a series of cooperating centers. The original bacterial system now includes the algae, yeasts, molds, protozoa, and hybridomas.

COOPERATING UNITS:
V. Jones, Food and Drug Administration
F. Benedict, Food and Drug Administration
R. Gherna, American Type Culture Collection
L. Blaine, American Type Culture Collection
R. Good, Centers for Disease Control
M. Segal, Environmental Protection Agency
L. Wayne, Veterans Administration
B. Kirsop, World Federation for Culture Collections
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Texas at San Antonio
V. Levy-Frebault, Pasteur Institute
A. Bussard, University of Nice
H. Sugawara, Institute for Physical and Chemical Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00250-11

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Algorithms for Microbial Systematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walczak, Cynthia	Computer Scientist	MSS, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR
Mercer, Paula	Computer Programmer	MSS, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, EODPP, NIDR

COOPERATING UNITS *(if any)*

See Attachment

LAB/BRANCH

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.37	2.37	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A program has been developed and is being used to compare and evaluate methods and/or laboratories when characterizing the same set of strains. The usual statistical packages are not useful because of the predominantly binary (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicate determinations), species, genus, and overall set for determination of test method equivalences and/or interlaboratory consistency. The program is being used to evaluate TB reference laboratories worldwide and to help develop new criteria for the separation of the AIDS patient-infective M. avium from the closely related noninfective M. intracellulare.

The first edition of the personal computer version of the Microbial Information System (MICRO-IS) was completed and is undergoing extensive testing in cooperating units. A program for conversion of controlled vocabulary information in text records of the Hybridoma Data Bank into the highly compressed, table oriented MICRO-IS format has been implemented. The conversion process also has extensive spelling, syntax, and format error checking capabilities. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required for computer database building of valuable archival paper records of phenotypic strain data.

Computer graphic algorithms are being tested to aid microbiologists in visualizing individual similarities as well as hierarchical group memberships among strains.

COOPERATING UNITS: V. Jones, Food and Drug Administration
F. Benedict, Food and Drug Administration
R. Gherna, American Type Culture Collection
L. Blaine, American Type Culture Collection
R. Good, Centers for Disease Control
M. Segal, Environmental Protection Agency
L. Wayne, Veterans Administration
B. Kirsop, World Federation for Culture Collections
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Texas at San Antonio
V. Levy-Frebault, Pasteur Institute
A. Bussard, University of Nice
H. Sugawara, Institute for Physical and Chemical Research

ANNUAL REPORT OF THE ANALYTICAL STUDIES BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

On May 2, 1988, the Analytical Studies Branch was created as a result of the reorganization of the Epidemiology and Oral Disease Prevention Program (EODPP). In the reorganization, the Epidemiology Branch was retained in the EODPP while the Science Transfer and Research Analysis Branch (STRAB) and the Disease Prevention Branch were dissolved. In their place, the Analytical Studies Branch and the Disease Prevention and Health Promotion Branch were created, thus maintaining the three branch structure of the EODPP. Formerly, the STRAB consisted of a staff of eight people. Four staff members from the STRAB comprise the Analytical Studies Branch as currently structured. The remaining staff of four from STRAB joined other personnel to form the Disease Prevention and Health Promotion Branch. The activities and accomplishments of these latter four persons for the 1988 fiscal year will be reported in the annual report of the Disease Prevention and Health Promotion Branch.

The Analytical Studies Branch conducts research analyzing the relationships among patterns of oral diseases and disorders, the need for and utilization of dental care, dental education, dental research, and dental delivery systems. These functions are carried out by: (1) investigating the interrelationships among ecomonic, social and personal characteristics, the distribution of various oral diseases, and dental treatment needed and utilized; (2) developing new models and theories which integrate social and epidemiological factors into models which explain the relations between oral disease and the social, economic, and personal characteristics of individuals; (3) conducting cost-effectiveness and cost-benefit analyses of various preventive and treatment methods and materials; and (4) providing consultation and technical assistance in these subject areas to other Institute components, the NIH, PHS, and other Federal and nonfederal scientists and organizations.

Analytical Studies

As a result of research conducted in fiscal year 1987 by three visiting scientists, Drs. Lawrence Meskin, Richard Oliver, and Leif Helöe, in concert with members of the Analytical Studies Branch, several manuscripts have been written based on that research. The papers have been prepared with the intent of advancing knowledge in dental epidemiology and have been submitted to journals for publication. The titles of the resultant manuscripts include:

> Periodontal diseases in the U.S. in 1981: prevalence, severity, extent, and role in tooth mortality.
>
> An estimate of periodontal treatment needs in the U.S. based on epidemiological data.
>
> A comparative study of costs for dental services and dentists´ incomes in the United States and Norway.

Dr. Allan J. Formicola, Dean of the Columbia University School of Dental and Oral Surgery, spent from October 1, 1987 through December 31, 1987 researching the relationship between nutrition and periodontal disease, namely the interrelationships between aging, nutritional status, and bone loss.

An interagency agreement, expected to be two years in duration, has been negotiated with the Boston Veterans Administration and the NIDR to study utilization, treatment needs, cost, and dental disease from a panel of healthy veterans. The purpose of the project is to collect and analyze data to study the relationships between dental disease and other oral conditions, treatment needs, economic factors, and utilization of dental services striving to hold other important variables constant. Utilization and cost data will be collected from private dental offices for the current panel of approximately 728 healthy males who have been examined periodically by the VA since 1968. This data will be merged with essential data previously collected as a component of the VA Dental Longitudinal Study to form an integrated analytical database. The NIDR and the VA will collaborate in the initial analysis of the collected data, and the NIDR will conduct additional analyses of the dataset.

National Health and Nutrition Examination Survey III (NHANES III)

Staff develped the diagnostic criteria for the restoration, tooth conditions, and prostheses assessment component of the oral clinical examination for the third cycle of the National Health and Nutrition Examination Survey (NHANES) in fiscal year 1987. Utilizing the final criteria, staff in fiscal 1988 provided the formal training and then assisted in the pilot testing of the clinical examiners at the Westat building prior to the deployment of the mobile examination centers to several test sites. Staff of the Analytical Studies Branch will assist in the NHANES III training and calibration scheduled for September 21-30, 1988, prior to the beginning of the survey shortly thereafter.

Other Activities

To disseminate knowledge about our research activities, presentations were made at major dental and economic meetings throughout the country. As an example, at the joint meeting of the International and American Associations of Dental Research in Montreal, Canada, these two presentation were made: Restorative and Prosthetic Treatment Needs in the U.S. in 1981 and Condition of the Dentition in U.S. Adults-1981. At the annual meeting of the American Public Health Association meeting in New Orleans, Louisiana, these two presentation were made: A New Approach to the Investigation of the Relationship Between Need and Demand for Dental Services and Potential Need and Demand for the Elderly Population. At the annual meeting of the American Association of Public Health Dentistry in Las Vegas, Nevada, the presentation, Patterns of Tooth Loss in the Elderly, was given. At the annual session of the Association of Military Surgeons of the United States, the presentation, Patterns of Tooth Loss in Employed Adults, was given. At the annual session of the Eastern Economic Association and at an invited seminar at the Univeristy of Chicago, the presentation, A New Theoretical Approach to the Demand for Dental Services, was given.

On May 25 in commemoration of the 40th anniversary of the National Institute of Dental Research, a symposium entitled Dental Science-Dental Health: The Status of American Oral Health was held in Washington, D.C. As a part of that three and one-half hour symposium, a member of the branch gave a presentation, Tooth Mortality and Patterns of Tooth Loss.

The Analytical Studies Branch took the lead in the computer-generation of graphics for presentation, such as slides and overheads, with the purchase of the software package, Harvard Graphics. The quality of slides for presentation at national meetings has been enhanced immensely by the use of this software in which the user develops the format such as a text chart, bar chart, or pie chart and choses a variety of colors for slides. The branch has also assumed the responsibility for exploring the marketplace for a reliable film recorder. A film recorder has been purchased and will enable staff from EODPP as well as the Extramural Program and the Research Data and Management Information Section to produce computer-generated slides quickly, easily, and much less expensively than has previously been possible.

Members of the Analytical Studies Branch served as referees for the scientific review of manuscripts submitted for consideration for publication for such journals as Gerodontics, Social Science and Medicine, and the Journal of Public Health Dentistry.

A member of the branch is the NIDR representative to the Federal Interagency Forum on Aging-Related Statistics which began meeting in October, 1986 to plan collaborative efforts to collect data on the aged. The forum was established to encourage cooperation among approximately 25 Federal agencies in the development, collection, analysis, and dissemination of data on the older population. Through cooperation and coordinated approaches, the forum intends to extend resources among agencies through joint problem solving, identification of data gaps, and improvement of the statistical information bases on the older population. The work of the forum will facilitate the exchange of information about needs at the time new data are being developed or changes are being made in existing data bases. Recently, two work groups have been formed. The first work group is reviewing the recommendations in the report of the National Research Council entitled The Aging Population in the Twenty-First Century: Statistics for Health Policy. The second work group is to think about a proposed longitudinal study on aging. The group will consider the data that will be needed in the future for such purposes as program planning, evaluation, and to support services for a changing elderly population and then develop a plan for the acquisition of the data.

Publications

Miller A.H., Brunelle J.A., Carlos J.P., Brown L.J., Loe H. Oral health of United States adults. The national survey of oral health in U.S. Employed Adults and Seniors: 1985-1986. National findings. NIH publication no. 87-2868, August 1987.

Meskin L.H., Brown L.J., Brunelle J.A., Warren G.B. Patterns of tooth loss and accumulated prosthetic treatment potential in U.S. employed adults and seniors, 1985-86, Gerodontics 4:126-135, 1988.

Brown, L.J., Meskin, L.H. Sociodemographic differences in tooth loss patterns in U.S. employed adults and seniors, 1985-86 Gerondontics 1988. (in press)

Brown, L.J. The long-run cost characteristics of dental practices in the U.S.A. Social Science and Medicine 1988. (in press)

Meskin, L.H., Brown, L.J. Prevalence and patterns of tooth loss in U.S. adult and senior populations, 1985-86. J. Dent Educ (Special Edition) 1988. (in press)

Brown, L.J. The economic outlook for medicine and dentistry. Medical Practice Management 1988. (invited paper)

Oliver, R.C., Brown, L.J., Loe, H. An estimate of periodontal treatment needs in the U.S. based on epidemiologic data. J. Perio. 1988. (in press)

Brown, L.J., Oliver, R.C., Loe H. Periodontal diseases in U.S. in 1981: prevalence, severity, extent, and role in tooth mortality. J. Perio. 1988. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00447-02 EODPP

PERIOD COVERED

October 1, 1987 - August 15, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Epidemiology of Periodontal Diseases and Implications for Treatment Needs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP NIDR

COOPERATING UNITS *(if any)*

University of Minnesota School of Dentistry

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.25	.20	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The study used epidemiological data on periodontal disease collected with a household survey of the U.S. population which describe the prevalence, severity and extent of periodontal diseases. These epidemiological data were then used to estimate the periodontal treatment needs of the U.S. as well as the time and dollars required to provide the treatment. Data from this study suggest that a decline in treatment methods have resulted in a reduction in periodontal treatment needs when compared with estimates from earlier epidemiologic studies. Only a small percentage of the population was found to have advanced periodontal disease, often involving only a few teeth, that required complex treatment. The vast majority of treatment needed was for the treatment of gingivitis, for prevention, and for the maintenance of periodontal health. When viewed from the perspective of expenditures, the annual cost to provide needed scaling and surgery to the entire population would be slightly less than 6% of the total expenditures for dental care in 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00472-01 EODPP

PERIOD COVERED

October 1, 1987 - May 1, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Patterns of Tooth Loss and Accumulated Treatment Potential in Employed Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Brunelle, Janet A.	Supvy. Statistician (Health)	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR
Oldakowski, Richard	Computer Programmer	EODPP NIDR

COOPERATING UNITS *(if any)*

Graduate School, University of Colorado Health Sciences Center

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.65	.50	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data from the National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986 were analyzed to introduce a new approach to the description of tooth loss patterns. A new typology was developed called tooth loss and prosthetic treatment (TLPT). In this typology, dental arches are classified into eight mutually exclusive, exhaustive categories. These categories relate the extent and location of missing teeth in the arch to one another and to the teeth that remain while providing some insight into the clinical options available to restore function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00473-01 EODPP

PERIOD COVERED

January 12, 1987 - March 14, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Restorative and Treatment Needs in the U.S. in 1981

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.20	.20	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Treatment needs based on the existence of dental clinical conditions are important determinants along with socioeconomic variables of the demand for dental services and dental manpower. Little information is currently available on the extent of needs in the U.S. This study reports the types and amount of treatment needed by the U.S. population in 1981 and provides an estimate (in 1985 dollars using ADA fees) of the expenditures required to treat those needs. From a household probability survey of 7700 persons in the U.S., information was collected on the condition (sound, filled, missing, etc.) and the needed treatment (filling, crown, extraction, etc.) of each permanent tooth. Information was also collected on type of prostheses (full, partial, fixed) present and needed for each permanent tooth. Selected findings follow: 43 percent of the U.S. population needed 1 or more restorations at the time of examination; 37 percent needed a posterior restoration while only 18 percent needed an anterior restoration; 9 percent needed an extraction; 2 percent needed endodontics; 4 percent needed an anterior crown; and 11 percent needed a posterior crown. The total cost in 1985 dollars to treat these conditions is estimated at slightly over 100 billion dollars. Close to 70 percent of the total is for the placement of new prostheses where none existed at the time of exam. Persons 55 years and older accounted for 30 percent of the cost while persons 35 to 54 accounted for 40 percent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00474-01 EODPP

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

National Health and Nutrition Examination Survey III - Tooth Conditions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR

COOPERATING UNITS *(if any)*

National Center for Health Statistics

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.30	.30	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The third National Health and Nutrition Examination Survey (NHANES III) is one of a series of studies conducted by the National Center for Health Statistics involving interview, physical examination, and diagnostic testing of a representative sample of Americans. Unlike other surveys in this series, this one has a longitudinal component. The selection procedure for the sample is designed to yield national estimates of diseases and health characteristics. Approximately 40,000 Americans in ramdomly selected communities are expected to be interviewed and 30,000 examined over two-three year sequential waves of data collection. The survey will begin in the fall of 1988. The NIDR is supporting this NCHS effort in several ways. The Analytical Studies Branch has prepared criteria for the assessment of restorations, tooth conditions, and prostheses - one of the more than 10 components of the dental examination of NHANES III. The data from the national survey will provide the first information on the prevalence and extent of dental conditions such as fractured restorations, gross loss of tooth structure, and defective prostheses. The inforamtion will be useful as a baseline for comparison with future surveys and will provide a basis for estimating the amount of dental services needed to treat these conditions.

these conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00475-01 EODPP

PERIOD COVERED

January 11, 1988 - September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

A Study of Utilization, Treatment Needs, Cost, and Dental Disease in Veterans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR

COOPERATING UNITS *(if any)*

Veterans Administration, Boston, Massachusetts
Harvard School of Dental Medicine

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
.35	.25	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The aim of this work is to compile a dataset that will be used to analyze the relationship between oral health measures, treatment needs as derived from those measures, and the utilization of specific dental services. Data will be collected from dental practices to measure the utilization and costs of dental services received during the past 12 years for the entire panel of 728 healthy veterans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00476-01 EODPP

PERIOD COVERED
March 28, 1988 - August 5, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Sociodemographic Differences in Tooth Loss Patterns

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Oldakowski, Richard	Computer Programmer	EODPP NIDR

COOPERATING UNITS *(if any)*
Graduate School, University of Colorado Health Sciences Center

LAB/BRANCH
Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.35	.25	.10

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Data for this work is derived from the Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986. This study identifies those sociodemographic groups at high risk for developing severe, function - compromising tooth loss of early onset and rapid development. Low income and less education appear to be the highest risk factors. Race appears to be intermediate and perhaps can be explained by income and educational levels. Gender differences in tooth loss are minimal.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00477-01 EODPP

PERIOD COVERED
January 19, 1988 - September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Condition of the Dentition in U.S. Adults - 1981

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.50	.30	.20

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This study used epidemiological data on dental conditions collected from a household survey of a national probability sample of the U.S. civilian population. These data were analyzed to identify the percent of teeth that were sound, satisfactorily filled, in need of fillings, missing, and finally missing and replaced by a prosthesis or missing and not replaced by a prosthesis in the dentitions of persons 18 years of age and older. The percent of sound teeth did not vary substantially with education; however, the percent of missing teeth was negatively correlated with education. Further, this analysis supports the trend in the improvement of the dental health of the public since the 1960's based on several dental conditions such as loss of teeth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00478-01 EODPP

PERIOD COVERED

October 1, 1987 - May 1, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Comparative Study of Costs for Dental Services in the U.S. and Norway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Chief	EODPP NIDR
Warren, Galen B.	Dental Research Analyst	EODPP NIDR

COOPERATING UNITS *(if any)*

Dental Faculty, University of Oslo

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.30	.10	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This work explores both the similiarities and differences between the United States and Norway in terms of the economies of the two countries and their dental care delivery systems.

PHS 6040 (Rev. 1/84) GPO 914-918

ANNUAL REPORT OF THE DISEASE PREVENTION AND HEALTH PROMOTION BRANCH NATIONAL INSTITUTE OF DENTAL RESEARCH

The Disease Prevention and Health Promotion Branch (DPHPB) is a new entity, officially created on May 2, 1988. The branch is comprised primarily of staff members from the Clinical Trials Section of the former Disease Prevention Branch (Dr. Driscoll, Ms. Nowjack-Raymer and Ms. Furnia) and certain members of the former Science Transfer and Research Analysis Branch staff (Dr. Gift, Ms. Horowitz, Dr. Houghton, Mr. Small and Mr. Zindel). In addition to new activities, the DPHPB has assumed responsibility for numerous activities ongoing under the previous organizational structure, including three direct operational clinical trials, all oral health education and health promotion activities, including those related to NIDR's role in a research and action program for improving the oral health of adults and older Americans, and the resource and promotion activities related to the fluoridation of community water supplies.

The Branch consists of two sections. At the present time, Dr. Driscoll is serving as Chief, Disease Prevention Section and as Acting Branch Chief. The position of Section Chief, Health Promotion Section has not yet been filled. The functions of the sections are:

Disease Prevention Section

(1) Plans and directs clinical trials, field studies and demonstrations of the effectiveness, feasibility and cost-effectiveness of accepted or new agents and procedures for the prevention of oral diseases; (2) Provides consultation, technical assistance, scientific issue resolution and other assistance to communities, organizations, institutions and individuals with regard to the efficacy, implementation and continuation of various oral disease prevention procedures and programs; (3) participates in collaborative research efforts with other Institute staff members.

Health Promotion Section

(1) Promotes the use of established effective preventive and therapeutic procedures by both the public and health professionals; (2) promotes the adoption of behaviors that are conducive to the improvement of oral health; (3) conducts research to identify and evaluate new materials and strategies for promoting oral health and to evaluate and improve existing materials and strategies; (4) participates in collaborative research and promotional efforts with other Institute staff members.

BRANCH ACTIVITIES

Disease Prevention Section

Concern has been expressed as to whether the ingestion of fluoride from various sources has increased in recent years with a concomitant increase in dental fluorosis. To address these concerns, a survey was conducted in 1985 among children in four areas of Illinois with water fluoride concentrations of

1, 2, 3, and 4 times the optimal, and these findings were compared with findings of a previous survey made in the same communities in 1980. Results showed that the distributions of fluorosis scores of young children (8-10 year olds) in 1980 and 1985 were quite similar. However, older children (13-15 year olds) in 1985 showed a greater prevalence and severity of fluorosis than did their cohorts in 1980. For example, in 1980 fluorosis scores involving staining and/or pitting affected more than 6% of the maxillary labial surfaces of teeth only in the four times optimal area, whereas, in 1985 all above-optimal areas showed at least 6% of these surfaces with severe forms of fluorosis. Despite the observed increase in fluorosis among older children, in the area with the optimal water fluoride concentration, the type of dental fluorosis detected was still limited to the less advanced forms (whitish blemishes of enamel) which have only cosmetic effects. As in 1980, the prevalence of dental caries continued to be markedly lower in all above-optimal fluoride areas compared with the optimal area. A final report, depicting the findings, was published in April 1988.

Several sources of fluoride have been identified as contributing to systemic fluoride ingestion by infants and young children. These sources include commercial infant formulas, fluoride dentifrices and dietary fluoride supplements prescribed in excess dosage or without knowledge of the amount of fluoride already present in the drinking water. However, a number of actions have been taken in recent years to limit fluoride intake from these sources. Consequently, any increase in the extent of fluorosis being reported now may prove transitory. Further prevalence surveys, similar to those conducted in Illinois, are needed to help monitor future levels of dental fluorosis among U.S. school children.

Further progress in the area of caries prevention is likely to come from use of certain combinations of fluoride procedures in conjunction with dental sealant therapy; fluorides are most effective in inhibiting decay in smooth surfaces of teeth, whereas sealants have proven effective in preventing pit and fissure decay. The most immediate challenge is to improve the methods of delivery of fluoride and sealants in ways that will enhance effectiveness and permit more cost-effective application to a greater proportion of the population at risk.

Results of an 11-year study in Nelson County, Virginia, which consisted of weekly rinsing in school with a 0.2% NaF solution, daily ingestion in school of a 1 mg fluoride chewable tablet and home use of a fluoride dentifrice, showed a 65% reduction in caries prevalence compared with baseline findings. Of the remaining decay, more than 90% occurred in pit and fissure tooth surfaces. Accordingly, in 1983, a sealant program was added to the ongoing fluoride program in an attempt to protect the highly susceptible pits and fissures in occlusal, lingual, and buccal surfaces. Newly erupted permanent teeth of children of selected ages received sealant therapy each year for a period of four years. Final results obtained in the fall of 1987, along with interim data, are currently being analyzed. The combined preventive program has the potential of virtually eliminating dental caries among Nelson County school children. Additionally, information has been obtained on a variety of factors that influence cost, including sealant retention, number of teeth treated, time required to carry out the procedure, salary of operators, and cost of materials.

The pronounced anticaries effects of the combined fluoride regimen in Nelson County are greater than those usually reported for any of the individual components, suggesting an additive effect. However, because all children in Nelson County received the entire regimen, no certain claims of additive effectiveness can be made. To specifically compare the combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone, a long-term clinical trial was initiated in 1981 in Springfield, Ohio. Study subjects at baseline comprised 1640 subjects in the first and second grades of 20 elementary schools. Interim two-year findings showed that the combined procedure produced a 33% lower increment in dmfs (primary teeth), and five-year interim findings showed a 31% lower increment in DMFS (permanent teeth) compared with the fluoride mouthrinse procedure alone, which may itself have had an effect in lowering dental caries. The tablet procedure alone also showed a lower caries score than the mouthrinse procedure, but the difference was not statistically significant. Interim results, therefore, indicate that the combined fluoride treatment does provide additive benefit beyond that which may have been derived from fluoride mouthrinsing alone. More definitive relationships among the three treatment groups should emerge as the study participants become older and continue to receive the treatments.

Although gingivitis does not necessarily always lead to the development of progressive periodontal disease, the reduction of gingivitis is still the best approach to the promotion of periodontal health. A reduction in gingivitis is best achieved through the use of effective mechanical oral hygiene measures. However, a number of studies have indicated that it is difficult to motivate children to maintain good oral hygiene over long periods of time when the emphasis is placed on removing dental plaque. Additional research has suggested that the elimination of gingival bleeding may produce better results than plaque control as a motivational tool for long term maintenance of optimal oral hygiene. To test this hypothesis, a two-year clinical trial was initiated in 1987 in York County, Virginia in two groups of ninth and tenth grade school children. Children in one group received instruction for the self-assessment of gingival bleeding, whereas those in another group received the traditional instructions in plaque control. Children in both groups are supplied with soft toothbrushes, wooden toothsticks for interdental cleaning, and detailed instructions in their proper use. An oral prophylaxis was given to all available participants at the end of the first year. Follow-up examinations will determine if any differences can be shown between the groups in their periodontal health. If the expected benefit in favor of the bleeding approach can be verified and sustained, the results have implications nationally for oral health education progress.

In contrast to the York County study, a chemical approach using stannous fluoride to prevent the development of plaque and reduce gingivitis was investigated under contract with the University of Minnesota (N01-DE-52556). Several animal and short-term human studies have demonstrated clear-cut antimicrobial effects for stannous fluoride, thus warranting renewed attention. Three randomly assigned groups of adults with gingivitis but without any bone loss (periodontal case Type I) brushed twice a day with either a 0.4% SnF_2 gel, a 0.22% NaF gel, or a placebo gel for a period of 18 months. Examinations were conducted at baseline and after six, twelve and eighteen months to assess the levels of gingivitis, bleeding, probing depth, attachment loss and staining for each of the study groups. A final report was submitted by the contractor on April 1, 1988. The findings showed that subjects using the SnF_2 gel had significantly more stain than individuals in the NaF or placebo groups at 6, 12 and 18 months. Statistically significant reductions in gingivitis and bleeding occurred in all three study groups over the course of the study. There were no statistically significant differences observed in gingivitis and bleeding in the SnF_2 or NaF groups when compared to the placebo group at 6, 12 or 18 months when applying an analysis of variance to the three groups. However, with pairwise comparison less gingivitis was observed in the SnF_2 group when compared to the placebo group at 12 months, and less bleeding was also observed in the SnF_2 group compared to the placebo group in good compliers at 12 months. The advantage of SnF_2 using pairwise comparisons at 12 months was not sustained at 18 months. Thus, the findings of this study are not encouraging with respect to the attainment of long-term benefits to gingival health with the use of SnF_2 gels applied with a toothbrush.

Community water fluoridation remains the most efficient and cost-effective method for preventing dental caries. Currently, about 63% of the U.S. population on public water supplies have access to fluoridated water. Staff of the Disease Prevention Section in FY88 provided technical assistance to the states of Pennsylvania, New York, Florida, Washington, Massachusetts, New Jersey and Montreal, Canada. During the NIDR 40th Anniversary celebration, staff was involved with the Grand Rapids Commemoration. Staff also reviewed numerous scientific documents regarding fluorides for a variety of public agencies and professional organizations.

Health Promotion Section

A study initiated and supported through a purchase order mechanism and conducted by Dr. John Newman of Georgia State University to inventory all existing national oral health and dental databases and assess their analytical potential both singly and in combination was completed in the winter, 1988. A workshop to discuss the implications of the findings for additional research related to the oral health initiative to reduce tooth loss was held in September 1988. The summary and conclusions of the workshop are being evaluated and prepared for distribution. The inventory of databases will be made available as an internal document.

A study initiated and supported through a purchase order mechanism and conducted by Ms. Bayley Milton to inventory existing oral health education and promotion activities for adults was completed. Telephone calls to approximately 500 selected national and state organizations were performed and copies of available oral health education and promotion materials were provided. Evaluation of the material and activities is being performed and a summary of the oral health programs and educational materials for adults is being prepared.

Staff have been active in developing and pretesting the dental component of the household interview of the third cycle of the NHANES III that will be conducted by the National Center for Health Statistics in the fall of 1988. Additionally, staff have been active in developing the dental and orofacial pain components of the household interview of the 1989 National Health Interview Survey that was pretested in June 1988 and will be conducted in 1989. Additionally, staff have been involved in the development of survey instruments for the International Collaborative Study, II.

Staff have continued to be active in developing a plan for a research and action program for improving the oral health of adults and older Americans. Using existing data sources, major oral health issues and key target groups are being developed based on observed needs. Individual, professional, and environmental interventions are being identified. Staff have been analyzing national data bases to describe dentally related attitudes and behaviors, as well as sociodemographic variables, in relation to oral health measures in an attempt to improve the targeting potential of the oral health promotion plan. The plan has been revised based on reviews by dental and health promotion experts. Four ad hoc working groups have provided expert input for the development of the overall plan or key components of it. In May 1988, as part of the 40th Anniversary of NIDR, this initiative to improve the oral health status of adults as an extension of efforts for children was announced. A specific emphasis is being placed on accelerating the reduction in tooth loss. NIDR has initiated meetings with other governmental agencies and private sector organizations to expedite the implementation of the plan. Meetings have been held with agencies such as the Centers for Disease Control (CDC), Health Resources and Services Administration (HRSA), National Center for Health Services Research (NCHSR), American Association for Dental Research, American Association of Dental Schools, and American Dental Association. Other agencies are being contacted. Both CDC and HRSA have assigned staff to work actively with NIDR in the initiation of this program.

Staff continues to provide consultation and assistance to educational and health agencies in the U.S. and abroad regarding oral disease prevention and health promotion. Staff collaborated with UNICEF and the University of Minnesota in the training and education of a dentist from Beijing Medical University, Beijing, China. The dentist, Dr. Hon Ying-Wang was sent to the University of Minnesota by UNICEF to learn about oral health education and promotion. Dr. Wang spent two weeks at the NIH working with DPHPB staff. Staff worked with the Veterans Administration, Centers for Disease Control, Food and Drug Administration, and the American Dental Association to develop educational materials on infection control for health care providers. The materials consist of videos and printed materials. One video will be premiered at the 1988 ADA annual session in Washington D.C. This project is an educational effort designed to foster the use of appropriate infection control measures in dental settings. A staff member was a member of the DHHS Region IX evaluation team sent to Hawaii to evaluate the state´s dental health section of their Department of Health. The Hawaii State Department of Health requested the evaluation from the Regional Administrator of Region IX. Staff also began work with Dupont on an oral health unit for their Worksite Wellness Program in which they will use two of NIDR´s educational films. Work was also done through the American Association of Public Health Dentistry´s Oral Health Committee to develop and distribute recommendations for teaching the use of pit and fissure sealants. A staff member worked with Migrant Health to organize a special session on oral health education for the 1988 Annual Migrant Health Conference held in Florida. Staff revised, reprinted and distributed two fluoride posters: "Give a Kid a Healthy Smile...Use Fluoride" and "Weekly Fluoride Rinses for a Healthy Smile". Staff continues to supervise the distribution of the program´s educational films, exhibits and printed materials.

Other DPHPB Activities

Staff have been active in numerous NIDR committees and working groups. One staff person is a member of the advisory group of the NIH/NIDR contract (86-309), "Assessment of NIH, Industry, and Academic Relationships in Restorative Materials Research", providing expertise in survey instrument design. Additionally, staff participated in the health services research working group convened through the Extramural Program.

Staff has also been active in working with other Institutes and agencies, providing health promotion and research expertise. Staff has also worked with the National Institute on Aging (NIA) on the evaluation and implementation of an Institute of Medicine proposal on health promotion and the older adult and served on special review groups to evaluate grant applications. Staff also served as a consultant on the National Cancer Institute´s Cancer Information System Marketing Plan Working Group and served as a reviewer for a NCHSR small grant. One staff member is participating as a member of a planning group for a 1989 meeting on oral health initiatives in maternal and child health, a HRSA Maternal and Child Health program. Staff has also participated in a WHO planning meeting entitled "Oral Manifestations of Human Immunodeficiency Virus Infection: Towards International Collaboration." Additionally, staff has organized and is participating in a panel on oral health promotion at the XIII World Conference on Health Education. One staff member is responsible for evaluation of scientific programs and membership services for the Federation Dentaire Internationale (FDI) as well as serving on several committees of that organization: Oral Health Promotion Working Group, Marketing Working Group and Scientific Program Committee. Another member serves on the Periodontal Health Working Group of the FDI. One staff member serves on the American Dental Association National Advisory Committee on Fluoridation. Another serves as scientific consultant to the ADA Council on Dental Therapeutics. A staff member also attended and participated in the Illinois State Workshop on Fluoridation for Waterworks Operators in December 1988 and participated in the National Oral Health Conference in Indianapolis in May 1988.

Several staff members participated in a week-long course entitled "Quantitative Methods in Epidemiology". One member completed the NIH Grants Associate Seminar Series. Staff have served as reviewers for manuscrupts submitted for publication to numerous journals, including the Journal of Dental Research, Social Science and Medicine, Health Education Quarterly, Journal of Public Health Dentistry, Community Dentistry and Oral Epidemiology, Advances in Dental Research and Social Sciences and Medicine (England).

Staff are also active in the broader NIH community. One staff member is NIDR´s representative to the NIH Women´s Health Issues Committee and the BID Prevention Coordinator´s Committee, and is alternate representative to the R&W Executive Council and is on the R&W Board of Directors, serving as corresponding secretary.

Publications

Heifetz, S.B., Driscoll, W.S., Horowitz, H.S. and Kingman, A. Prevalence of Dental Caries and Dental Fluorosis in Areas with Optimal and Above-Optimal Water-Fluoride Concentrations: A 5-Year Follow-up Survey. Journal of the American Dental Association. 116:490-495, April 1988.

Loupe, M.J., Frazier, P.J., Horowitz, A.M., Kleinman, D.R., Caranicas, P.C., and Caranicas, D.H. Impact of an NIDR Educational Program on the Teaching of Caries Prevention in Dental Hygiene. Journal of Dental Education. 52:149-155, 1988.

Gift, H.C. Prevention of Dental Caries - The Summary of Community Professional and Individual Efforts. Evaluation of Health Professions. 10:323-41, 1987.

Gift, H.C. Health Promotion and Aging--Dental Health, Surgeon General's Workshop on Health Promotion and Aging, B1-B20, 1988.

Gift, H.C. Attitudes and Behaviors of Health Professionals Toward Older Adults, module 3, American Association of Dental Schools Geriatric Education Project. 1988; A3:1-11.

Dionne, R.A., Gift, H.C. Drugs Used for Parenteral Sedation in Dental Practice, Anesthesis Progress. 35:199-205, 1988.

Gift, H.C. The Awareness and Assessment of Periodontal Problems Among Dentists and the Public, International Dental Journal. 38:147-153, 1988.

Gift, H.C. Issues of Aging and Oral Health Promotion, Gerodontics. 4:194-206, 1988.

Horowitz, A.M. and Frazier, J. Promoting Oral Health Among Older Americans: The Role of Primary Prevention. Journal of Family and Community Health. 11:33-41, 1988.

Presentations

Gift, H.C. The Awareness and Assessment of Periodontal Problems Among Dentists and the Public, Annual World Dental Congress of the Federation Dentaire Internationale, October, 1987.

Gift, H.C., Kleinman, D.V. Dental Health Attitudes and Behaviors of the United States Public, Annual Meeting of the American Public Health Association, October, 1987.

Horowitz, A.M. The Role of Fluorides in Dental Caries Prevention. University of Hawaii, January 15, 1988, Honolulu, Hawaii.

Horowitz, A.M. and Frazier, P.J. Strategies for Effective Community Oral Health Programs, four hour workshop presented at the Yankee Dental Congress, January 23, 1988, Boston, MA.

Horowitz, A.M. Implementing Appropriate Community-based Oral Health Programs. Department of Health Ecology, School of Dentistry, University of Minnesota, March 3, 1988, Minneapolis, MN.

Dionne, R., Gift, H.C. Survey Techniques and Drugs Used for Parenteral Sedation in Dental Practice, presented at the 66th General Session of the International Association for Dental Research, March, 1988.

Frazier, P.J., Loupe, M.J., Horowitz, A.M. and Kleinman, D.V. Faculty Need for Caries Prevention Teaching Resources, International Association for Dental Research, Montreal, Canada, March, 1988.

Heifetz, S.B., Nowjack-Raymer, R., Driscoll, W.S. and Li, S.-H. Evaluation of the Additive Effectiveness of Fluoride Tablets and Fluoride Mouthrinsing: Interim Results After 5 years, 66th General Session of the International Association for Dental Research, March, 1988.

Horowitz, A.M. Education of the Public. NIDR AIDS Task Force Meeting. March 15, 1988.

Horowitz, A.M. The Appropriate Use of Fluorides in Community-based Programs. Georgetown University, Department of Operative Dentistry, School of Dentistry, Georgetown University, Washington, D.C., March 31, 1988.

Horowitz, A.M. School-based Caries Preventive Procedures, Department of Community Dentistry, University of Pennsylvania, Philadelphia, PA, April 21, 1988.

Horowitz, A.M. School-based Health Promotion Activities to Prevent Dental Caries. NIH BID Prevention Coordinators Meeting, NIH Building 1, NIH, May 10, 1988.

Horowitz, A.M. Mechanical Plaque Removal: An Update. Postgraduate Seminar, Howard University, Washington, D.C., May 12, 1988.

Gift, H.C. A National Oral Health Promotion Plan, presented at the XIII World Conference on Health Education, August, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00070-16 EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988 CT DE 720045

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Combined self-applied fluorides and sealants for caries prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Driscoll, William S.	Acting Chief, Disease Prevention and Health Promotion Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.62	.52	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In October 1972, a self-administered dental health program was started in Nelson County, Va., a fluoride-deficient community. Children in the County's schools, under teacher supervision, chewed and ingested daily a 1 mg F tablet and rinsed weekly with a 0.2% NaF solution. A fluoride dentifrice was provided for ad libitum use at home. Baseline DMFS examinations were made of 2,138 children in the County's elementary (grades 1-6), junior (grades 7 and 8), and senior (grades 9-12) high schools. Follow-up DMFS examinations were conducted at two-to-three year intervals until 1983 when the full effectiveness of the program was achieved.

In the fall of 1983, a sealant program was added to the ongoing fluoride program. Children who were 6, 7, 12, and 13 were eligible to have pit-and-fissure sealants applied. An initial screening to identify those tooth surfaces to be sealed was made in December 1983. Caries data (DMFS) from the September 1983 dental examination will serve as a baseline for those children who participate in the sealant phase of the study. In succeeding years, new groups of 6 and 12 year olds have been enrolled. Treatments have continued for four years. Interim dental examinations took place at the start of the third year of the study (September 1985) and final examinations were made in September 1987. Following data analysis final reports will be prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00310-08 EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988 CT DE 810144

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Driscoll, William S.	Acting Chief, Disease Prevention and Health Promotion Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.41	.14

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In September 1981, a self-administered dental program was begun in Springfield, Ohio, a fluoride-deficient community. The approximately 1700 children attending 20 public and non-public elementary schools were randomly assigned to one of three treatment groups. The children in Group I dissolve and ingest daily a 1 mg F tablet; the children in Group III rinse weekly with a 0.2% NaF solution; and Group II carries out both procedures. The assigned treatments are self-administered under the supervision of the teacher.

Before the procedures were started, baseline examinations were conducted. First and second follow-up dental examinations were conducted in October 1983 and November 1986 respectively. The procedures will be continued through 1989 and followed by a clinical examination. Three to four years following the cessation of treatment, a post-treatment examination will be conducted in high school to determine the extent of continued protection.

Supplies have been ordered, schedules arranged and local personnel identified for the start of the eighth school year of treatment in October 1988. Clinical examinations will be conducted in May 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00396-04 EODP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Caries, fluorosis, gingivitis and calculus in areas with different water-fluoride levels.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Driscoll, William S.	Acting Chief, Disease Prevention and Health Promotion Branch	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Disease Prevention Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involved the same seven communities in Illinois that had been previously surveyed by the NIDR in April 1980. The communities were grouped according to the relation of their water fluoride concentration for the area (1, 2, 3, or 4-times optimal). Study participants consisted of children in two age groups, 8-to-10 and 13-to-15 years of age. The 8-to-10 year age group comprised children who received parental consent to participate and whose histories indicated that they had resided continuously since birth in their respective communities. The 13-to-15 year old group comprised only those children who were examined as 8-to-10 year olds in 1980. Examinations for the survey were conducted in April 1985. Dental caries was assessed with the DMFS index and fluorosis was measured with the Tooth Surface Index of Fluorosis, an index developed by NIDR for the 1980 survey. These indices were applied in both age groups by the same examiners who had used them in 1980. A new examiner assessed the prevalence of gingivitis and calculus in the entire dentition in the 13-to-15 year age group. A report presenting findings on dental caries and fluorosis has been published.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00439-02 EODPP

PERIOD COVERED
October 1, 1987 to September 30, 1988 ~~CT DE 870170~~

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
An evaluation of different approaches to prevent gingivitis in teenage children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nowjack-Raymer, Ruth E.	Clinical Trials Specialist Disease Prevention and Health Promotion Branch (DPHPB)	EODPP, NIDR
Driscoll, William S.	Acting Chief (DPHPB)	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Disease Prevention Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.56	.38	.18

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In April 1987 a study was begun to evaluate two approaches to the prevention of gingivitis in teenage children. Baseline examinations for periodontal health (plaque, pocket depth, calculus, gingival bleeding), DMFS and gingival recession were conducted on 500 ninth and tenth graders in the York County High Schools, Virginia. Questionnaires regarding oral hygiene methods and professional care practices were completed by each student. Following the examinations, subjects were randomly assigned by grade to either a positive control or test group.

The control group received a manual for the self-assessment and control of plaque and the test group received a manual for the self-assessment and control of gingival bleeding. Every subject participated in small group sessions for instruction in the self-assessment procedures and supply distribution. Two weeks later, individual instruction was held to ensure that the procedures were understood.

Examinations of periodontal health were conducted in October and April of FY'88. In May 1988 each participant was given the opportunity to have an oral prophylaxis and all participants received individual instruction to reinforce the appropriate group methods.

Periodontal health status will be assessed in October 1988 and April 1989. DMFS and gingival recession examinations will be repeated at the final examination in April 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00444-02

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Health Attitudes and Dentally Related Behaviors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen	Sociologist	EODPP, NIDR
Kleinman, Dushanka	Special Asst. to Associate Director for Program Coordination	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	ASB, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.31	.30	.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study uses survey and interview data collected from a household sample of the U.S. population and describes the associations among attitudes, behaviors and oral health. The data analyses are used in the oral health promotion plan, papers and publications in preparation and will assist in understanding and improving the oral health of individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00453-02

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Analysis of Existing Oral Health Data on National Surveys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen	Sociologist	EODPP, NIDR
Brown, L. Jackson	Economist	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch, EODPP

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.08	.08	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The potential value of existing data sources for further analyses is being investigated utilizing a purchase order mechanism. National dental survey and oral clinical exams conducted over the past 30 years have been identified and contacts have been made with sponsors and principal investigators to determine the status of data files. Reviews of publications and other mechanisms are being used to determine the appropriateness of additional work, with emphasis on possible trend analysis and interpretation for public policy. A workshop to discuss the existing data sources and make recommendations for future analyses was held in September, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00463-01

PERIOD COVERED

May 1, 1988 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Inventory of Existing Oral Health Education and Promotion Activities for Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen	Sociologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The potential value of existing oral health education and promotion activities for adults is being investigated utilizing a purchase order mechanism. Telephone calls to approximately 500 selected national and state organizations using standardized interview format has provided a description of oral health education and promotion activities being performed. Copies of available oral health education and promotion materials have been provided and evaluations to determine more extensive use of materials and programs are being performed. A summary of the available programs and educational materials is being prepared as well as a summary of the evaluation.

ANNUAL REPORT OF THE EPIDEMIOLOGY BRANCH NATIONAL INSTITUTE OF DENTAL RESEARCH

A major activity of the Epidemiology Branch during FY 1988 was the analysis of data and reporting of results of the 1986-1987 National Survey of the Oral Health of U.S. School Children. It was demonstrated that the decline in caries, first reported in 1981, had continued at approximately a constant rate. Children aged 5-17 years averaged about 3 decayed, missing or filled permanent tooth surfaces, over 60 percent fewer than in the early 1970′s. A remarkable finding from the recent survey was that 50 percent of the children examined had never experienced caries in the permanent dentition. Although the cause of this major decrease in a once endemic disease is not known with certainty, it is assumed that fluoride, in its various forms of delivery, has played a central role.

These data will be published shortly in monograph form, as will the geographic region-specific results of a survey of the oral health of U.S. adults and elderly, completed in 1986.

Using data from the 1987 survey of caries in children, two additional studies have begun. One involves analysis of differences in caries levels in children who have been lifelong residents of fluoridated or non-fluoridated communities. The second is concerned with the possible relationship of extremes of caries experience (high caries vs. caries free) to certain waterborne trace elements in the local environment.

Final examinations were completed in a longitudinal study of the incidence of incipient peridontal disease in Navajo Indian adolescents. Analyses of interim data collected from this population showed that periodontal attachment loss was significantly associated with the site-specific presence of subgingival calculus, gingival bleeding and Bacteroides intermedius. The findings provide further evidence for the belief that this microorganism may be an important pathogen in the initiation of chronic periodontitis.

A study of the natural history of periodontal disease in Sri Lankan tea laborers is continuing. Three subpopulations have been identified based on rates of periodontal disease destruction and tooth loss due to periodontal disease. Bacteriological, immunological, radiographic, and genetic studies of these populations is under way. The results will improve understanding of the natural occurrence of periodontal disease.

A cooperative study with dental research in Norway also continues. Databases are being established which will allow comparison with U.S. data of factors which may influence the destruction of the periodontium. Studies are focusing on the stability of gingival lesions over time and their conversion to a progressive loss of attachment.

The newly organized Soft Tissue, Craniofacial Defects and Pain Section initiated research in several areas which have received little prior epidemiologic study including oral mucosal lesions, traumatic injuries to teeth, and the oral manifestations of HIV infection. Staff of the Branch are collaborating with personnel at the Perry Point, Maryland Veteran′s Administration Hospital to document the occurrence of soft-tissue lesions by examinations of 1000 inpatients. In addition data on soft-tissue lesions as well as histories of use of tobacco and alcohol were collected as a part of the 1986-1987 survey of The Oral Health of School Children. Preliminary analyses have shown the prevalence of these lesions in 12 to 17 year old subjects to be around 4%, most of which were smokeless tobacco-associated white lesions.

In collaboration with staff at the Walter Reed Army Medical Center, who are implementing a longitudinal study of the National History of HIV Infection, a dental component has been developed and incorporated into the research protocol.

Approximately 1000 HIV infected military personnel will be followed by periodic oral examinations to document the incidence of oral lesions and disorders in relation to the stage of HIV infection, and to study the role of saliva in the transmission of the virus. Staff have also participated in national and international workshops to develop recommendations for research on the oral aspects of AIDS.

To begin to document the epidemiology of traumatic injuries to teeth and the face, an inventory of available databases was prepared. These vary widely in quality and completeness, but permitted preliminary analyses of product-related dental injuries from 1979 to 1987. Dental injuries tended to increase during this period. Children below age 15 sustained over 75 percent of all reported injuries, which most often occurred during summer. Dental injuries were also documented during the initial clinical examinations in a longitudinal study in Bogalusa, La. Of 777 junior and senior high school children, 24 percent had evidence of traumatic injury to teeth.

Using data from the National Hospital Discharge Survey, a study was done of fractures of the skull and facial bones from 1981 to 1985. Results showed that reported fractures had declined during this period. Males, and especially those aged 15-44, constituted the highest risk group for these injuries.

Staff of the Branch provided consultation and assistance to various organizations in connection with the planning and initiation of epidemiologic research. These included the National Center for Health Statistics (the Branch is coordinating the dental component of NHANES III, and has developed an automated data entry system for the collection of the clinical data), the Republic of Ireland, Federal Republic of Germany, University of New Mexico, Louisiana State University, University of Hong Kong and several state and local dental public health directors.

Publications

Carlos, J.P., Brunelle, J.A. and Wolfe, M.D. Attachment Loss vs. Pocket Depth as Indicators of Periodontal Disease: A Methodologic Note. J Perio Res 22:524-525, 1987.

Loupe M.J., Frazier, P.J., Horowitz, A.M., Kleinman, D.V.,et al. Impact of an NIDR Educational Program on the Teaching of Caries Prevention in Dental Hygiene. J Dental Educ 52:149, 1988.

Bhat, M. and Nelson, K.B. Developmental Enamel Defects in Primary Teeth in Children with Cerebral Palsy, Mental Retardation, or Hearing Defects. A Review: In Proceedings of the Workshop on Developmental Defects of Enamel, Rotorua, New Zealand, 1988, Advances in Dental Research.

Meskin, L.H., Brown, L.J, Brunelle, J.A. and Warren, G.B. Patterns of Tooth Loss and Accumulated Prosthetic Treatment Potential in U.S. Employed Adults and Seniors. 1985-86. Gerondontics 4:126-135, 1988.

Carlos, J.P., Wolfe, M.D., Zambon, J.J. and Kingman, A. Periodontal Disease in Adolescents: Some Clinical and Microbiological Correlates of Attachment Loss. J Dent Res. In press.

Carlos, J.P. and Wolfe, M.D. Dental Caries. Historic and Current Perspectives. Compend Contin Educ Dent. In press.

Carlos, J.P. and Wolfe, M.D. Methodologic and Nutritional Issues in Assessing the Oral Health of the Aged. Am J Clin Nutr. In press.

Presentations

Carlos, J.P. Dental Caries: Historic and Current Perspectives. Symposium on Fluoride Usage. University of Pennsylvania.

Carlos, J.P. Periodontal Disease in Adolescents. NIDR Clinical Associate Seminar.

Brunelle, J.A. Extent and Severity of Root Surface Lesions; and Introduction and Background of the Adult Survey, AAPHD Symposium on Oral Health of Older Americans, October 1988.

Brunelle, J.A. Tooth Mortality in the U.S. Association of Military Surgeons of the United States Annual Meeting, November 1987.

Swango, P.A. Dental Caries Findings from the NIDR Adult Survey. Association of Military Surgeons of the United States Annual Meeting. Las Vegas, November, 1987.

Kleinman, D.V. Oral Soft Tissue Research Directions. Association of Military Surgeons of the United States Annual Meeting. Las Vegas, November, 1987.

Swango, P.A. Dental Caries in U.S. Adults. Dental Caries Working Group, National Adult Oral Health Promotion Program, Bethesda, February, 1988.

Brunelle, J.A. Root Caries: Distribution by Tooth and Surface Type in U.S. Adults, IADR Meeting, Montreal, Canada, March 1988.

Swango, P.A. Epidemiology of Dental Caries. Lecture for Seniors, Georgetown University School of Dentistry. Washington, March, 1988.

Swango, P.A. Summary and Update of the Walter Reed HIV Project. NIDR AIDS Task Force Meeting. Bethesda, March, 1988.

Swango, P.A. NHANES III: Data Collection Plans for Baby Bottle Tooth Decay. Oral Health Subcommittee of Healthy Mothers/Healthy Babies. Washington, March, 1988.

Swango, P.A. Dental Caries in U.S. Adults. Annual ADA Dental Student Research Conference. Bethesda, April, 1988.

Kleinman, D.V. Overview of the Epidemiology and Oral Disease Prevention Program and Preparing for and Establishing Careers in Dental Research. Annual ADA Dental Student Research Conference. Bethesda, April, 1988.

Carlos, J.P. Dental Caries in U.S. Children. 1986-87. NIDR 40th Anniversary Symposium, May 1988.

Brunelle, J.A. Regional Findings from Adult Survey. NIDR 40th Anniversary Symposium, May 1988.

Kleinman, D.V. and Swango, P.A. Epidemiology of Oral Conditions. Presentation to MPH class of 1988. Uniformed Services University of the Health Sciences. Bethesda, June, 1988.

Kleinman, D.V. 1986-87 NIDR Survey of Oral Health in U.S. Schoolchildren: Assessment Procedures for Smokeless Tobacco Use and Oral Health Effects. Workshop of NCI Grantees for Behavioral Interventions for Control of Smokeless Tobacco Use. Rockville, June, 1988.

Bhat, M. and Li, S-H. Incidence of Fractures of the Skull and Face, United States, 1981-85. NCHS Data Users Conference. Bethesda, July, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00399-04

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Periodontal Diseases in Adolescents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carlos, James P.	Chief, Epidemiology Branch, NIDR, EODPP
Wolfe, Mary D.	Epidemiologist, NIDR, EODPP

COOPERATING UNITS *(if any)*

Department of Oral Biology, SUNY at Buffalo

LAB/BRANCH

Epidemiology Branch

SECTION

Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	1.2	.55

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this continuing study is to determine the prevalence and progression of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in the U.S.

The original cross-sectional study population consisted of approximately 600 Navajos, ages 14-19 years. Twenty-four posterior interproximal sites were examined on each subject. Gingivitis was assessed using a modification of the G.I. Loss of attachment was assessed using Ramfjord's technique. Bone loss was diagnosed from standardized bite-wing radiographs.

Analyses indicated a high prevalence of disease: gingivitis (71%); attachment loss (89%); and bone loss (89%). The average number of sites in the mouth affected with the more advanced form of disease (attachment loss and bone loss) was also high: 32% of the sites had attachment loss, and 22% had bone loss. A longitudinal study is in progress with the youngest subjects to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease. Two hundred twenty-six 9th and 10th grade students were examined in February 1986, 1987 and 1988 using the same clinical and radiographic techniques. Subgingival plaque samples were obtained from all first molars and analyzed for A. actinomycetemcomitans, B. gingivalis and B. Intermedius. Analyses of first year data indicate that the combination of calculus, gingival bleeding and B. intermedius gave the most parsimonious explanation of the presence of attachment loss. Analyses of the longitudinal data is progressing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00403-04

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Collection and Analysis of Oral Health of U.S. Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR
Miller, Ann J.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology

SECTION

Dental Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.35	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Survey of Oral Health in U.S. Adults was designed to establish the prevalence of coronal caries, root surface caries and periodontal destruction in a readily accessible adult population with sufficient precision to permit future detection of changes by geographic region and within five year age intervals. The sampling frames for this survey included U.S. business establishments listed by Standard Industrial Codes (SIC) and maintained by Dun & Bradstreet and county rosters of multipurpose senior centers compiled from lists confirmed by the state and local area agencies on aging. The primary sampling units were counties, the second stage sampled business establishments or senior centers, and the third-stage sampling unit were the employees or seniors. The categories of Agriculture and Mining, the military, the permanently unemployed, and persons who are not employed outside the home were excluded from the sample.

Both samples were stratified into seven geographic regions of the contiguous 48 states. For employed persons the sample was also stratified by urban/rural, mean income and percent minority in the sampled counties and by size of business establishments. The final sample consisted of 15,132 persons aged 18-64, representing approximately 100 million employed adults in these age groups, and of 5686 retired persons, aged 65-80+, representing 4 million seniors.

The survey was conducted under contract with Westat Inc., of Rockville, Maryland. The data were processed by the Biometry Section, Epidemiology Branch, NIDR. The detailed findings are presented in two monographs. "Oral Health of U.S. Adults, National Findings: NIH #872868 and Oral Health of U.S. Adults, Regional Findings: NIH #882869.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00420-03

PERIOD COVERED
Ocotber 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Design and Analysis of National Survey of Oral Health in School Children

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR
Miller, Ann J.	Epidemiologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Dental Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	.50	3.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

A National Survey of the Oral Health of School Children was implemented in 1986. A probability sample of approximately 52,000 school-aged children in Kindergarten through 12th grade was selected. Thirteen examiners were trained and calibrated by NIDR staff in criteria for measurement of coronal caries, periodontal disease, fluorosis and presence of lesions of the oral soft tissues. Dental exams on approximately 41,000 children were conducted during the 1986-1987 school year by the 13 dental teams. A questionaire on use of smokeless tobacco and alcohol was also administered and a salivary sample was collected. The overall response rate for participation was 78%. Data processing of forms and questionaires was done.

Preliminary analysis of the national estimates of DMF permanent surfaces indicated a 37% change from mean DMFS estimates from a similar study conducted in 1979-80. There was a lower level of disease at every age. Mean DMFS was also lower in all regions of the country; however, there were still differences between regions as before. Approximately 50% of the children ages 5-17 were caries free in their permanent dentition. A monograph on dental caries prevalence is being prepared. Data analysis will continue during the coming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-000432-02

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Relationship of Bone Loss in Adolescents to Periodontal Status in Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carlos, James. P.	Chief, Epidemiology Branch,NIDR, EODPP
Wolfe, Mary D.	Epidemiologist, NIDR, EODPP

COOPERATING UNITS *(if any)*

University of Umea
Umea, Sweden

LAB/BRANCH
Epidemiology Branch

SECTION
Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.40	.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this investigation was to determine whether periodontal destruction, diagnosed as an adult, can be predicted by examining bite-wing radiographs taken in adolescence.

This retrospective investigation was conducted in Sweden where a unique system of lifetime address registry and public dental care records are available for cross-referencing and locating subjects. The university of Umea identified 250 subjects, approximately 30 years of age, for whom bite-wing radiographs taken at age 15 were still available, and who resided within a short distance from Umea. Dental examinations were conducted in November on 240 subjects at four dental clinics in the surrounding county of Vasterbotten. Clinical components consisted of examines for evidence of gingival bleeding, calculus, pocket depth, and attachment loss at the buccal and mesio-buccal aspects of all teeth excluding third molars. Bite-wing radiographs were taken using an Eggen film standardizing device. Also, a brief questionnaire regarding past periodontal treatment was administered.

Assessment of alveolar bone level is being conducted with the aid of video analysis software (JAVA) which allows for enhancement of the image through contrast manipulation, background subtraction, and a variety of filtering systems (smoothing, median, and convolution).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DE00468-01

PERIOD COVERED
Ocotber 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dental Caries in U.S. Children in Fluoridated/Non-Fluoridated Areas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR
Smith, Jacqueline I.	Statistician (Health)	PS, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Dental Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Residential histories collected during a national survey of oral health of U.S. schoolchildren conducted in 1986-1987 will be used to establish two groups: those children who always lived on a public water supply in a fluoridated community and those who never lived in a fluoridated community.

Comparisons of the mean levels of Decayed, Missing and Filled Surfaces will be made by age, sex and region of the country. Differences in percent of caries free individuals and dental caries levels for deciduous teeth in the two groups will also be analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE00469-01

PERIOD COVERED
October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Distribution of Root Caries in U.S. Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Dental Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1985 the NIDR conducted a national survey of the oral health status of employed adults and seniors, in which measurements of root caries were made for the first time on a national sample of the U.S. population. Examinations were made on 15,132 employed adults representing 99.6 million persons. Previous reports of the distribution of root caries vary widely, which may be related to how root caries was defined or measured. For the survey, visual tactile examination were made for decayed and filled root surfaces. Each tooth was considered to have four root surfaces. No x-rays were taken.

Approximately 21% of the employed dentate population aged 18-64+ years had at least one decayed (D) or filled (F) root surfaces (S) with more males than females affected. The mean number of DFS was less than one for the employed dentate population. Less than half (47%) of the DFS were filled.

Distribution of the disease by tooth and surface type was analyzed. Buccal surfaces were the most frequently affected surfaces with decay or fillings occuring four times as often here as on any other surface. The distal and mesial surfaces are the next most frequent sites of root caries and the lingual surface is the least affected. However, the differences among these last three surface types is minimal. Root caries was most common in mandibular pre-molars, molars and maxillary cuspids. Lower incisors were rarely involved (<1% of the DFS occurred in these teeth).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01- DE-00470-01

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Demographic and Environmental Correlates of High and Low Caries Experience in Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Carlos, James P.	Chief, Epidemiology Branch, NIDR, EODPP
Wolfe, Mary D.	Epidemiologist, NIDR, EODPP

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION
Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.50	.20

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the striking findings of the 1986-1987 NIDR Survey of Dental Caries of U.S. School Children was that approximately half the children ages 5-17 had never experienced caries in their permanent teeth. This study is exploring the relationship between a caries-free status and such variables as sex, ethnicity, geographic region and urban-rural residence. Controls are the approximately 1400 children of the same ages whose cumulative caries experience exceeds two standard deviations of the mean (mean DMFS) for that age group.

Preliminary analyses indicate highly significant differences among the two groups with respect to geographic region of residence. Therefore the study is being expanded to include data on ten trace metals obtained from drinking water samples from the schools attended by the subjects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00410-04

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Natural History of Periodontal Disease in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Löe, Harald	Director, NIDR	PDS, EODPP, NIDR
Smith, Jacqueline I.	Statistician	PDS, EODPP, NIDR

COOPERATING UNITS *(if any)*

University of Texas Dental School, San Antonio, Texas

LAB/BRANCH

Epidemiology Branch

SECTION

Periodontal Diseases Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study of the natural history of periodontal disease in Sri Lankan tea laborers is continuing. Three sub-populations have been identified, based on rates of periodontal disease destruction and tooth loss due to periodontal disease: (1) ~10% of the group had rapid progression (RP) and extensive tooth mortality; (2) ~10% showed no progression (NP) of the disease and (3) ~80% showed moderate progression (MP) of periodontal destruction.

Bacteriological, immunological, radiographic and genetic studies of the three populations are under way.

The specific aims of these studies are to compare the presence and levels of selected putative periodontal pathogens, peripheral blood antibody titers and to evaluate the genetic determinants in the three groups.

Building of two master data sets in SAS for the longitudinal Norwegian surveys 1968-1985 (15) was completed. Data bases are: LOE.NOR.AVO (all valid observations) and LOE.NOR.IAS (in all surveys). An additional set of data has been received extending the Norwegian survey thru 1988. The observations are being assimilated into the previous databases.

Continued studies have focused on the stability of the gingival lesion, the mean number of years needed for an inflamed gingival unit to convert to progressive loss of attachment and the factors which may influence the continuity and discontinuity of the destruction of the periodontium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-00429-02 -EODPP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Periodontal Health and Oral Soft Tissue Lesions in Adolescent Population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mohandas Bhat, Visiting Scientist, EB, EODPP, NIDR
Carla Bock, Computer Programmer, EODPP, NIDR

COOPERATING UNITS *(if any)*
Louisana State University, Mussleman, R.J. and Gardiner, J.E.
School of Dentistry, New Orleans, Louisana, Cassingham, R.J.; Dummett, C.O.
and Schneider, P.E.

LAB/BRANCH
Epidemiology Branch

SECTION
Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this longitudinal study are: 1) to determine whether early periodontal lesions (attachment loss and horizontal bone loss) indicate a predisposition to chronic periodontitis, 2) to determine the relationship between the prevalence of various types of oral mucosal lesions and patterns of consumption of alcohol, cigarette and smokeless tobacco, and 3) to determine the prevalence and causes of fractured anterior teeth, in an adolescent population.

The baseline data on prevalence of caries and periodntal lesions has been collected, and progress is being made in the analysis of these data.

The analysis of the data on consumption of alcohol, cigarette, and smokeless tobacco, among these adolescents showed that smokeless tobacco usage is a predominantly white male activity, and decreases after 15 years of age. Alcohol use and smoking, however, transcends all groups with increasing usage with increasing age.

Analysis of data on traumatized anterior teeth showed that clinical evidence of injury was found in 24% of the children, whose injured teeth were distributed as follows: dentine involvement 73%, unrestored with discoloration 13%, restored 5%, endodontic therapy 2%, and lost teeth 7%. Although there was an overall agreement of 75% between anamnestic and clinical data, in those children with clinical evidence of injury only 45% had a self-reported injury. Conversely, in those with self-reported injury only 47% showed confirming clinical evidence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00430-02-EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Product-Related Injuries to Teeth, Mouth, and Face

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mohandas Bhat, Visiting Scientist, EB, EODPP, NIDR
Shou-Hua Li, Statistician (Health), EODPP, NIDR

COOPERATING UNITS *(if any)*

Consumer Product Safety Commission, Bethesda, Maryland

LAB/BRANCH

Epidemiology Branch

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this study is to do a trend analysis of product-related, hospital emergency room-treated data on injuries to the teeth, mouth and face, including an analysis of the frequencies of such injuries related to specific consumer products, on data compiled by the National Electronic Injury Surveillance System (NEISS) maintained by the Consumer Product Safety Commission.

The NEISS database has separate data on injuries to the teeth, mouth and face. The injuries to the teeth form a subset of mouth injuries. During this reporting period the subset of injuries to teeth was updated to to include data for 1986 and 1987, and the entire data set for the years 1979 through 1987 was analyzed.

Findings showed that the projected national estimates of yearly dental injuries during this nine-year period ranged from 9,623 to 20,840. There was a slight but consistent trend in increase in dental injuries from 1979 through 1986. More males than females were injured each year in all age groups, except for those over 65 years of age, although their numbers were small. Children under 5 years accounted for 40.42 percent and those from 5 to14 years of age accounted for 34.93 percent of the injuries. Thus, over 65 percent of the dental injuries were sustained by children below 15 years of age. The aggregated data showed the peak incidence of dental injuries during the month of May; with May, June and July showing the highest incidence. The most common products/activities associated with dental injuries were riding bicycles, falling down stairs or steps, baseball, hitting or falling from tables, desks, chairs or beds, basketball, football, and bathtubs/showers. These were followed by hard particles in the food causing injury to teeth, swings/swing sets, slides/sliding boards and skateboards.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00443-02-EODPP

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Prevalence of Oral Soft Tissue Lesions in Patients in a Long-Term Care Facility

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Swango, Philip A.	Dental Epidemiologist	EB, EODPP, NIDR
Kleinman, Dushanka V.	Chief, Soft Tissue, Craniofacial Defects and Pain Section	EODPP, NIDR

COOPERATING UNITS *(if any)*

Perry Point VA Medical Center
Perry Point, Maryland

LAB/BRANCH

Epidemiology Branch

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is a cross-sectional survey to document oral soft tissue pathologies occurring in patients presenting to the Perry Point VA Medical Center during a 12-month period. It is expected that about 1,000 patients will be examined during the study. Pathologies that cannot be definitively diagnosed by the examining dentist will be referred to a consultant oral pathologist. The objectives of the study are to estimate the prevalence of oral soft tissue lesions in this patient population, to characterize the range and severity of the pathologies noted and to assess treatment needs resulting from these conditions. Information will also be collected from existing records regarding risk factors such as dental prostheses, medical condition, medications, and use of tobacco and alcohol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00464-01

PERIOD COVERED

September 1, 1988 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Oral Manifestations of HIV Infection in a U.S. Military Population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Philip A. Swango,D.D.C., EODPP, NIDR
Dushanka V. Kleinman, D.D.S., EODPP, NIDR
Philip Fox, D.D.S., CIPCB, IRP, NIDR
Carolyn Tylenda, D.D.S., CIPCB, IRP, NIDR
Joseph Zambon, D.D.S., SUNY at Buffalo
Edmund Tramont, MD, Walter Reed Army Medical Center
Charles Oster, MD., Walter Reed Army Medical Center

COOPERATING UNITS *(if any)*

Walter Reed Army Institute of Research

LAB/BRANCH

EB, EODPP, NIDR

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

United States Army personnel who have been tested as seropositive for the Human Immunodeficiency Virus (HIV) will be given medical examinations and treatment at Walter Reed Army Medical Center (WRAMC). These subjects will also be given the opportunity to participate in a medical research protocol to study the natural history of HIV infection, administered by the Walter Reed Army Institute of Research (WRAIR - see attached protocol). An oral component has been developed by staff of the National Institute of Dental Research (NIDR) in collaboration with U.S. Army medical and dental staff at WRAMC and WRAIR.

Under the existing WRAIR natural history portocol, the oral examination is limited to the identification of pseudomembranous candidiasis (thrush), hairy leukoplakia, and the detection of enlarged lymph nodes in the head and neck region. The addition of the oral component will permit documentation of the prevalence and incidence of the full range of oral conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions will also be characterized, and the role of oral manifestations as early predictors or markers of disease progression will be elucidated. Finally, the role of saliva in the transmission of the virus will be investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00465-01-EODPP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Facial Trauma Databases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mohandas Bhat, Visiting Scientist, EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epidemiologic data on the occurrence and distribution of oral and facial injuries and associated risk factors are needed in order to develop appropriate preventive strategies. Although published data on this topic are very sparse, there are several ongoing national and regional injury surveillance systems that collect useful data on oral and facial trauma. For several reasons, including the tendency of each agency to collect information suited to its own needs, these sources of data are variable with respect to their purposes, scope and methods of collection, and cannot easily be interpreted.

The objectives of this project are to examine the methodology, types of data collected, strengths and weaknesses of several public-domain and private databases from which information on oral and facial trauma may be gleaned. These include the National Electronic Injury Surveillance System, National Health Interview Survey, National Hospital Discharge Survey, National Accident Sampling System, Supplementary Data System of the Bureau of Labor Statistics, and surveillance systems of certain Athletic and Sports Associations. Based on a preliminary analysis of the available data, capsule information on each of these data systems was prepared and distributed to participants taking part in the annual meeting of the Assication of State and Territorial Dental Directors (ASTDD) and the National Oral Health Conference, held this year. The ultimate goal of this project is to determine the overall magnitude, characteristics and trends in oral and facial trauma, in the United States, pieced together from these various surveillance systems, in order to help develop appropriate preventive strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00466-01-EODPP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Skull and Facial Injuries From the National Hospital Discharge Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Mohandas Bhat, Visiting Scientist, EB, EODPP, NIDR
Shou-Hua Li, Statistician (Health), EODPP, NIDR

COOPERATING UNITS *(if any)*
National Center for Health Statistics

LAB/BRANCH
Epidemiology

SECTION
Soft Tissue, Craniofacial Defects and Pain Sections

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to do a trend analysis of the data on first listed diagnosis of fractures of the skull & face bones (S & F fractures) from the National Hospital Discharge Survey, conducted by the National Center for Health Statistics, for the years 1981 through 1985.

These data are collected on patients discharged from a national sample of short-stay general and specialty hospitals in the U.S. The data are coded according to ICD, 9th revision. Although detailed data on individual ICD codes are published yearly by the NCHS, the analysis undertaken here is previously unpublished. Results showed a declining trend in the estimated numbers of S & F fractures (ICD codes 800-803), which varied from 158,000 in 1981 to 120,000 in 1985. This paralleled, somewhat, the trend in estimated numbers of all fractures (ICD codes 800-829). S & F fractures as a percent of total fractures varied from 13.92 in 1981 to 10.59 in 1985. Although number of all fractures were approximately equal between the sexes, males had consistently more than twice as many S & F fractures as females. The age distribution of S & F fractures, was also consistent and was approximately as follows: 65% were in the age group of 15-44 yrs, 20% under 15yrs, 9% 45-64 yrs and 6% over 65 yrs. This study has shown that although there is a declining trend in the incidence of S & F fractures, males generally, and those between the ages of 15 and 44 years are in the high risk groups.

=======

INDEXES

=======

NATIONAL INSTITUTE OF DENTAL RESEARCH - ANNUAL REPORT FY88

INDEX BY PROJECT NUMBER

0 Z01 DE00001-36	FOLK, JOHN E.	126
0 Z01 DE00009-27	MARTIN, GEORGE R.	215
0 Z01 DE00012-26	FOWLER, BRUCE O.	108
0 Z01 DE00024-22	BROWN, KENNETH S.	216
0 Z01 DE00025-22	YAMADA, YOSHIHIKO	217
0 Z01 DE00028-21	HAND, ARTHUR R.	160
0 Z01 DE00031-20	BROWN, FREDERICK J.	283
0 Z01 DE00034-20	SIRAGANIAN, REUBEN P.	242
0 Z01 DE00042-18	CHASSY, BRUCE M.	264
0 Z01 DE00043-18	DONKERSLOOT, JACOB A.	265
0 Z01 DE00044-18	KRICHEVSKY, MICAH I.	337
0 Z01 DE00046-17	WAHL, SHARON M.	243
0 Z01 DE00049-17	CHUNG, SOO IL	128
0 Z01 DE00061-17	SANDBERG, ANN L.	266
0 Z01 DE00065-17	WEBBER, RICHARD L.	186
0 Z01 DE00070-16	DRISCOLL, WILLIAM S.	361
0 Z01 DE00074-16	FISHER, LARRY W.	109
0 Z01 DE00088-15	EANES, EDWARD D.	110
0 Z01 DE00132-14	HARGREAVES, KENNETH M.	284
0 Z01 DE00133-14	GRACELY, RICHARD H.	285
0 Z01 DE00134-14	HASCALL, VINCENT C.	111
0 Z01 DE00149-14	HASSELL, JOHN R.	219
0 Z01 DE00157-13	TORCHIA, DENNIS A.	112
0 Z01 DE00199-12	OLIVER, CONSTANCE	244
0 Z01 DE00204-12	REDDI, A. HARIDARA	130
0 Z01 DE00211-12	WEBBER, RICHARD L.	187
0 Z01 DE00212-12	WEIFFENBACH, JAMES M.	162
0 Z01 DE00230-12	KLEINMAN, HYNDA K.	220
0 Z01 DE00246-11	DIONNE, RAYMOND A.	286
0 Z01 DE00250-11	WALCZAK, CYNTHIA A.	339
0 Z01 DE00254-11	CISAR, JOHN O.	245
0 Z01 DE00273-10	KOLENBRANDER, PAUL E.	267
0 Z01 DE00275-10	MARTIN, GEORGE R.	221
0 Z01 DE00276-10	GRACELY, RICHARD H.	287
0 Z01 DE00282-09	MIRTH, DALE B.	325
0 Z01 DE00286-09	DIONNE, RAYMOND A.	288
0 Z01 DE00288-09	RUDA, MARYANN T.	289
0 Z01 DE00290-09	SIRAGANIAN, REUBEN P.	246
0 Z01 DE00291-09	DUBNER, RONALD	290
0 Z01 DE00310-08	HEIFETZ, STANLEY B.	362
0 Z01 DE00311-08	PARK, M.H.	132
0 Z01 DE00329-07	DUBNER, RONALD	291
0 Z01 DE00332-07	ROBERTS, MICHAEL W.	163
0 Z01 DE00336-07	BAUM, BRUCE J.	165
0 Z01 DE00337-07	FOX, PHILIP C.	166
0 Z01 DE00341-07	THOMPSON, JOHN	268
0 Z01 DE00366-06	MAX, MITCHELL B.	292
0 Z01 DE00373-06	WEBBER, RICHARD L.	188
0 Z01 DE00374-06	PLUZNIK, DOV H.	247
0 Z01 DE00377-05	KENSHALO, JR., DANIEL R.	293
0 Z01 DE00379-05	YOUNG, MARIAN F.	113
0 Z01 DE00380-05	ROBEY, PAMELA G.	114
0 Z01 DE00382-05	ROBRISH, STANLEY A.	269

0	Z01	DE00392-05	SMITH, PHILLIP D.	248
0	Z01	DE00396-04	DRISCOLL, WILLIAM S.	363
0	Z01	DE00399-04	CARLOS, JAMES P.	373
0	Z01	DE00403-04	BRUNELLE, JANET A.	374
0	Z01	DE00410-04	LOE, HARALD	380
0	Z01	DE00411-03	WRIGHT, WILLIAM E.	168
0	Z01	DE00412-03	BRAHIM, JAIME S.	169
0	Z01	DE00413-03	BENNETT, GARY J.	294
0	Z01	DE00414-03	IADAROLA, MICHAEL J.	295
0	Z01	DE00415-03	TURNER, ROY J.	171
0	Z01	DE00417-03	MIRTH, DALE B.	326
0	Z01	DE00418-03	KINGMAN, ALBERT	327
0	Z01	DE00420-03	BRUNELLE, JANET A.	375
0	Z01	DE00421-03	ROONEY, JAMES F.	309
0	Z01	DE00423-03	PRABHAKAR, BELLUR S.	310
0	Z01	DE00424-03	MC CARTNEY FRANCIS, NANCY	249
0	Z01	DE00429-02	BHAT, MOHANDAS	381
0	Z01	DE00430-02	BHAT, MOHANDAS	382
0	Z01	DE00431-02	YANAGISHITA, MASAKI	115
0	Z01	DE00432-02	CARLOS, JAMES P.	376
0	Z01	DE00433-02	ROBEY, F.A.	134
0	Z01	DE00434-02	ROBEY, F.A.	136
0	Z01	DE00435-02	KINGMAN, ALBERT	328
0	Z01	DE00436-02	LI, SHOU HUA	329
0	Z01	DE00437-02	ROBEY, F.A.	138
0	Z01	DE00438-02	AMBUDKAR, INDU S.	172
0	Z01	DE00439-02	HEIFETZ, STANLEY B.	364
0	Z01	DE00440-02	HYLDEN, JANICE L.K.	296
0	Z01	DE00441-02	ALLEN, JANICE	250
0	Z01	DE00443-02	SWANGO, PHILIP A.	383
0	Z01	DE00444-02	GIFT, HELEN	365
0	Z01	DE00447-02	BROWN, L. JACKSON	345
0	Z01	DE00453-02	GIFT, HELEN	366
0	Z01	DE00454-02	LONDON, JACK	270
0	Z01	DE00455-01	ROBERTS, MICHAEL W.	173
0	Z01	DE00456-01	WAHL, LARRY M.	251
0	Z01	DE00457-01	WELLNER, ROBERT B.	174
0	Z01	DE00458-01	KOUSVELARI, ELENI E.	175
0	Z01	DE00459-01	CHUDLER, ERIC	297
0	Z01	DE00460-01	MAHIN, RICHARD	298
0	Z01	DE00461-01	KINGMAN, ALBERT	330
0	Z01	DE00462-01	LI, SHOU HUA	331
0	Z01	DE00463-01	GIFT, HELEN	367
0	Z01	DE00464-01	SWANGO, PHILIP A.	384
0	Z01	DE00465-01	BHAT, MOHANDAS	385
0	Z01	DE00466-01	BHAT, MOHANDAS	386
0	Z01	DE00467-01	CASALI, PAOLO	312
0	Z01	DE00468-01	BRUNELLE, JANET A.	377
0	Z01	DE00469-01	BRUNELLE, JANET A.	378
0	Z01	DE00470-01	CARLOS, JAMES P.	379
0	Z01	DE00471-01	ABRAMCZUK, JAN W.	314
0	Z01	DE00472-01	WARREN, GALEN B.	346
0	Z01	DE00473-01	WARREN, GALEN B.	347
0	Z01	DE00474-01	WARREN, GALEN B.	348
0	Z01	DE00475-01	WARREN, GALEN B.	349
0	Z01	DE00476-01	WARREN, GALEN B.	350
0	Z01	DE00477-01	WARREN, GALEN B.	351
0	Z01	DE00478-01	WARREN, GALEN B.	352

NATIONAL INSTITUTE OF DENTAL RESEARCH - ANNUAL REPORT FY88

INDEX BY INVESTIGATOR NAME

0 Z01 DE00471-01	ABRAMCZUK, JAN W.	314
0 Z01 DE00441-02	ALLEN, JANICE	250
0 Z01 DE00438-02	AMBUDKAR, INDU S.	172
0 Z01 DE00336-07	BAUM, BRUCE J.	165
0 Z01 DE00413-03	BENNETT, GARY J.	294
0 Z01 DE00429-02	BHAT, MOHANDAS	381
0 Z01 DE00430-02	BHAT, MOHANDAS	382
0 Z01 DE00465-01	BHAT, MOHANDAS	385
0 Z01 DE00466-01	BHAT, MOHANDAS	386
0 Z01 DE00412-03	BRAHIM, JAIME S.	169
0 Z01 DE00031-20	BROWN, FREDERICK J.	283
0 Z01 DE00024-22	BROWN, KENNETH S.	216
0 Z01 DE00447-02	BROWN, L. JACKSON	345
0 Z01 DE00403-04	BRUNELLE, JANET A.	374
0 Z01 DE00420-03	BRUNELLE, JANET A.	375
0 Z01 DE00468-01	BRUNELLE, JANET A.	377
0 Z01 DE00469-01	BRUNELLE, JANET A.	378
0 Z01 DE00399-04	CARLOS, JAMES P.	373
0 Z01 DE00432-02	CARLOS, JAMES P.	376
0 Z01 DE00470-01	CARLOS, JAMES P.	379
0 Z01 DE00467-01	CASALI, PAOLO	312
0 Z01 DE00042-18	CHASSY, BRUCE M.	264
0 Z01 DE00459-01	CHUDLER, ERIC	297
0 Z01 DE00049-17	CHUNG, SOO IL	128
0 Z01 DE00254-11	CISAR, JOHN O.	245
0 Z01 DE00246-11	DIONNE, RAYMOND A.	286
0 Z01 DE00286-09	DIONNE, RAYMOND A.	288
0 Z01 DE00043-18	DONKERSLOOT, JACOB A.	265
0 Z01 DE00070-16	DRISCOLL, WILLIAM S.	361
0 Z01 DE00396-04	DRISCOLL, WILLIAM S.	363
0 Z01 DE00291-09	DUBNER, RONALD	290
0 Z01 DE00329-07	DUBNER, RONALD	291
0 Z01 DE00088-15	EANES, EDWARD D.	110
0 Z01 DE00074-16	FISHER, LARRY W.	109
0 Z01 DE00001-36	FOLK, JOHN E.	126
0 Z01 DE00012-26	FOWLER, BRUCE O.	108
0 Z01 DE00337-07	FOX, PHILIP C.	166
0 Z01 DE00444-02	GIFT, HELEN	365
0 Z01 DE00453-02	GIFT, HELEN	366
0 Z01 DE00463-01	GIFT, HELEN	367
0 Z01 DE00133-14	GRACELY, RICHARD H.	285
0 Z01 DE00276-10	GRACELY, RICHARD H.	287
0 Z01 DE00028-21	HAND, ARTHUR R.	160
0 Z01 DE00132-14	HARGREAVES, KENNETH M.	284
0 Z01 DE00134-14	HASCALL, VINCENT C.	111
0 Z01 DE00149-14	HASSELL, JOHN R.	219
0 Z01 DE00310-08	HEIFETZ, STANLEY B.	362
0 Z01 DE00439-02	HEIFETZ, STANLEY B.	364
0 Z01 DE00440-02	HYLDEN, JANICE L.K.	296
0 Z01 DE00414-03	IADAROLA, MICHAEL J.	295
0 Z01 DE00377-05	KENSHALO, JR., DANIEL R.	293
0 Z01 DE00418-03	KINGMAN, ALBERT	327
0 Z01 DE00435-02	KINGMAN, ALBERT	328

0	Z01	DE00461-01	KINGMAN, ALBERT	330
0	Z01	DE00230-12	KLEINMAN, HYNDA K.	220
0	Z01	DE00273-10	KOLENBRANDER, PAUL E.	267
0	Z01	DE00458-01	KOUSVELARI, ELENI E.	175
0	Z01	DE00044-18	KRICHEVSKY, MICAH I.	337
0	Z01	DE00436-02	LI, SHOU HUA	329
0	Z01	DE00462-01	LI, SHOU HUA	331
0	Z01	DE00410-04	LOE, HARALD	380
0	Z01	DE00454-02	LONDON, JACK	270
0	Z01	DE00460-01	MAHIN, RICHARD	298
0	Z01	DE00009-27	MARTIN, GEORGE R.	215
0	Z01	DE00275-10	MARTIN, GEORGE R.	221
0	Z01	DE00366-06	MAX, MITCHELL B.	292
0	Z01	DE00424-03	MC CARTNEY FRANCIS, NANCY	249
0	Z01	DE00282-09	MIRTH, DALE B.	325
0	Z01	DE00417-03	MIRTH, DALE B.	326
0	Z01	DE00199-12	OLIVER, CONSTANCE	244
0	Z01	DE00311-08	PARK, M.H.	132
0	Z01	DE00374-06	PLUZNIK, DOV H.	247
0	Z01	DE00423-03	PRABHAKAR, BELLUR S.	310
0	Z01	DE00204-12	REDDI, A. HARIDARA	130
0	Z01	DE00332-07	ROBERTS, MICHAEL W.	163
0	Z01	DE00455-01	ROBERTS, MICHAEL W.	173
0	Z01	DE00433-02	ROBEY, F.A.	134
0	Z01	DE00434-02	ROBEY, F.A.	136
0	Z01	DE00437-02	ROBEY, F.A.	138
0	Z01	DE00380-05	ROBEY, PAMELA G.	114
0	Z01	DE00382-05	ROBRISH, STANLEY A.	269
0	Z01	DE00421-03	ROONEY, JAMES F.	309
0	Z01	DE00288-09	RUDA, MARYANN T.	289
0	Z01	DE00061-17	SANDBERG, ANN L.	266
0	Z01	DE00034-20	SIRAGANIAN, REUBEN P.	242
0	Z01	DE00290-09	SIRAGANIAN, REUBEN P.	246
0	Z01	DE00392-05	SMITH, PHILLIP D.	248
0	Z01	DE00443-02	SWANGO, PHILIP A.	383
0	Z01	DE00464-01	SWANGO, PHILIP A.	384
0	Z01	DE00341-07	THOMPSON, JOHN	268
0	Z01	DE00157-13	TORCHIA, DENNIS A.	112
0	Z01	DE00415-03	TURNER, ROY J.	171
0	Z01	DE00456-01	WAHL, LARRY M.	251
0	Z01	DE00046-17	WAHL, SHARON M.	243
0	Z01	DE00250-11	WALCZAK, CYNTHIA A.	339
0	Z01	DE00472-01	WARREN, GALEN B.	346
0	Z01	DE00473-01	WARREN, GALEN B.	347
0	Z01	DE00474-01	WARREN, GALEN B.	348
0	Z01	DE00475-01	WARREN, GALEN B.	349
0	Z01	DE00476-01	WARREN, GALEN B.	350
0	Z01	DE00477-01	WARREN, GALEN B.	351
0	Z01	DE00478-01	WARREN, GALEN B.	352
0	Z01	DE00065-17	WEBBER, RICHARD L.	186
0	Z01	DE00211-12	WEBBER, RICHARD L.	187
0	Z01	DE00373-06	WEBBER, RICHARD L.	188
0	Z01	DE00212-12	WEIFFENBACH, JAMES M.	162
0	Z01	DE00457-01	WELLNER, ROBERT B.	174
0	Z01	DE00411-03	WRIGHT, WILLIAM E.	168
0	Z01	DE00025-22	YAMADA, YOSHIHIKO	217
0	Z01	DE00431-02	YANAGISHITA, MASAKI	115
0	Z01	DE00379-05	YOUNG, MARIAN F.	113

*U.S.GOVERNMENT PRINTING OFFICE:1989-241-243:93414

[illegible]
[illegible]

NIH Library, Building 10
National Institutes of Health
Bethesda, MD 20892

LIBRARY
NIH
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

INFORMATION CONSERVATION INC.

MAR 1989

NIH LIBRARY
3 1496 00368 5099